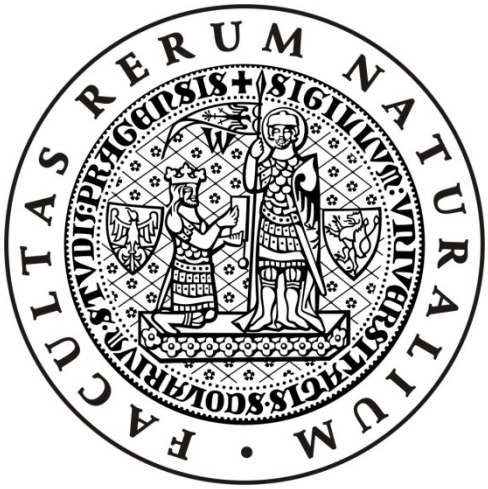
Univerzita Karlova v Praze, Přírodovědecká fakulta
Katedra ekologie

Charles University in Prague, Faculty of Science
Department of Ecology

Doktorský studijní program: Ekologie
Ph.D. study program: Ecology



Ing. Jana Knappová

Co určuje rozšíření trávníkových druhů na bývalých polích: struktura krajiny, podmínky prostředí, druhové vlastnosti nebo náhoda?

Landscape structure, habitat properties, species traits or chance: What determines distribution of grassland plants in abandoned fields?

Disertační práce
Ph.D. Thesis

Školitelka/Supervisor: Doc. RNDr. Zuzana Münzbergová, Ph.D.

Praha, 2012

Prohlášení /Declaration:

Prohlašuji, že tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu. Závěrečnou práci jsem zpracovala samostatně, s využitím dostupných zdrojů a můj podíl na článcích je vyjádřen pořadím mezi autory. Všechny použité informační zdroje a literatura jsou řádně citovány.

I declare that this thesis has not been submitted for the purpose of obtaining the same or any other academic degree earlier or at another institution. My involvement in the research presented in this thesis is expressed through the authorship order of the included publications and manuscripts. All literature sources I used when writing this thesis have been properly cited.

V Praze, 12. 8. 2012

Acknowledgements

This thesis would never exist without the immense support of my family. Especially, I would like to thank to Michal for endless and stimulating debates and for all imaginable kind of help including logistic, physiotherapy and catering. Klára often cured my bad mood from numerous failures and made me take science less seriously. And they both survived weeks at home with awfully biting invisible monsters hidden in seeds of experimental plants, they accompanied me during the field work regardless of sun or freeze, wind or storms.

My parents always did everything what they could and even more to help me with all that strange things I had to do. My mother Věra and also Jarka and Karel Knappovi took care of Klára and thus provided me time to work. I am aware that this is by far not commonplace to all grandparents.

I am grateful to my supervisor Zuzana Münzbergová for the main idea of this work, for trusting me without knowing me in advance and for countless critical comments and advices. I appreciate that I could share part of my work with Lucka Hemrová, who was always open to debates and ready to find solution on emerging problems. Other colleagues from department of Population Ecology at Institute of Botany provided comments and suggestions on early manuscript drafts. I also have to thank to Dana Průchová, who provided most of the data on species traits.

Katka Kadlecová, Adéla Kovaříčková, Mikuláš Michalík, Hedvika Nutilová, Honza Popelka, Katka Školníková, Roman Školník, Jana Švantnerová, Karel Výborný, Eva Warausová and Denis Waraus spent hours collecting, clearing, weighing and counting seeds or digging and weeding experimental plots and sowing seeds. Anežka Hradečná, Vendula Hrstková and Dana Parysová helped me with field measurements and recording of data. And after exhausting fieldwork, we always found comfortable accommodation in Litoměřice thanks to Borek Franěk. I am also grateful to Ilona Chmelařová who provided me a warm and friendly working place at Institute of Botany in the first months when I was stranger there and she was always ready to help me with all kind of technical stuff.

Financial support during my studies was provided by the Grant Agency of the Czech Republic (projects no. P505/10/0593, P504/10/0456 and doctoral project no. 206/08/H049).

Contents

Abstract

Semi-natural grasslands are among the most threatened habitats in Europe, endangered mainly by cessation of former management practices and conversion into other types of land use. Extensive research has been carried out in European grasslands in last decades, to explain origins of diversity and to provide guidelines for its conservation. However, the very slow response of perennial plants to landscape changes often impedes to accurately evaluate why species occur just where they occur and not elsewhere. Abandoned fields are perceived as alternative habitats for species from declining grasslands. Indeed, many species are able to spontaneously colonise abandoned fields, but many other species are absent from communities that develop there. An important question remains what limits their successful establishment. By answering this question, we can gain also important insights into factors determining species distribution in grasslands because colonization of recently abandoned fields by grassland species is the ongoing process which is not obscured by historical changes in landscape structure. The very basic aim of this thesis was to evaluate the *status quo* of dry grassland plants in fields abandoned in last two decades. And in the second step, to identify what are the main constrains of successful colonization, both at the level of environment and of species traits.

I proved that abandoned fields in the study region could be successfully colonized by species that are also common in dry grasslands (Chapter 1 and 4). For these species, abandoned fields likely represent suitable habitat. However, rare dry grassland species occur only sporadically in these fields and mainly in edges only. These species are presumably much more restricted to dry grasslands, either due to narrow habitat requirements or poor dispersal, and their conservation therefore relies on proper management of their current habitats. According to seed addition experiment (Chapter 2) the probability of establishing a viable and fertile population is largely constrained by the vigour of resident vegetation. The effect of seasonal variability emphasizes the necessity of temporal replication of seed addition experiments.

I have successfully tested novel approach for measuring habitat isolation, using distance and species richness of source habitats (Chapter 1). I have also shown that inclusion of habitat characteristics when testing for the effect of habitat isolation might considerably alter conclusions. I therefore recommend using both tests with and without inclusion of habitat characteristics in the future. I believe that these methodological improvements might push forward our understanding of factors that influence species richness not only in grasslands but also in other communities of perennial plants restricted to fragmented habitats.

In general, I found that species colonization success can be only hardly predicted based on traits related to dispersal, habitat requirements or competitive ability. The best predictor I found was niche width (Chapter 3), which also partly correlated with species frequency in source grasslands. The latter was by far the most strongly related to species frequency in abandoned fields (Chapter 3) and also to species ability to equally colonize field edges and interiors (Chapter 4). I think that it is unlikely that despite a wide range of traits under study there are still some very influential hidden traits that determine species abundance in both source and target habitats. Rather, this tight relationship between species frequency in dry grasslands and in abandoned fields implies some kind of positive feedback and indirectly confirms the role of neutral mechanisms in community assembly.

I have assessed number of factors that influence colonization process, both from environmental and species perspective. Nevertheless, the study system of dry grasslands and abandoned fields still offer challenges for future research and provide opportunities for testing interesting ecological hypotheses.

Introduction

Factors limiting plant species distribution

Understanding factors affecting species distribution is fundamental challenge in ecology. The question why some species occur somewhere and not elsewhere is very complex and can be seen from different complementary perspectives. First, abiotic environment provides basis for all further processes such as colonization, competition or reproduction (Myers & Harms, 2009). Abiotic environment involves a large spectra of factors from truly abiotic ones such as moisture, nutrient availability, light intensity, etc., across landscape level factors comprising habitat isolation to factors introduced by humans (such as disturbance management) or other organisms. The latter represent transition to another important determinant of species distribution - biotic interactions. Interactions between organisms might be both interspecific and intraspecific, positive or negative (Callaway & Walker, 1997). To makes it even more complicated, the nature of interactions might switch from positive to negative in time (e.g., with changes in life cycle of involved organisms; Aguiar & Sala, 1994; Farrer & Goldberg, 2011) or along environmental gradients (Choler *et al.*, 2001; Veblen, 2008; Holmgren & Scheffer, 2010). Many organisms are also able to influence back their environment (Jones *et al.*, 1997; Kylafis & Loreau, 2011), sometimes creating a loop of positive or negative feedbacks (Ganade & Brown, 2002; Vlasáková *et al.*, 2009; van de Voorde *et al.*, 2012). Third, the extent to which individual species is constrained or favoured by environmental conditions or by interactions with other organisms, is given by its inherent properties - species traits (Diaz et al., 1998; McGill et al., 2006; Violle et al., 2009).

The interplay among biotic and abiotic environment and species traits constitutes basis for niche-based community assembly (Shipley *et al.*, 2006; Kraft *et al.*, 2008). However, the effort to predict species occurrences based on species traits and environmental filtering might be unsuccessful due to dispersal limitation (Ozinga *et al.*, 2005) resulting in species absence from suitable habitats (Turnbull *et al.*, 2000; Münzbergová & Herben, 2005). In plants, the inability to reach suitable habitat could be partly caused by dispersal traits, such as seed mass, terminal velocity, adhesive potential, etc. (Tremlová & Münzbergová, 2007; Schleicher *et al.*, 2011) but can also remain unexplained (Walker *et al.*, 2006).

More recently, in (theoretical) communities of species with identical life-history characteristics and no adaptation to environmental niches, limited dispersal alone was also shown to be responsible for spatial distribution patterns similar to those found in nature (Hubbell, 2001). This started-up debate on whether patterns we can see in natural communities could be structured by stochastic, neutral mechanisms (Volkov *et al.*, 2003). Relative consensus has been done that both niche and neutral processes act in community assembly and their relative importance differs among communities (Tilman, 2004; Gravel *et al.*, 2006).

European grasslands and their specifics

Semi-natural grasslands are particularly important sources of biodiversity as they host not only a vast number of plant species (Pärtel *et al.*, 2005) but also vertebrate and invertebrate fauna (Hopkins & Holz, 2005). Most grasslands have developed as a consequence of pastoral agriculture being imposed on cleared woodland or drained marshland, or to natural climax grasslands modified by human activity but which still retain a predominance of native species and remain relatively ‘unimproved’ in agricultural terms (Emanuelsson, 2008; Hopkins, 2009). Over the past century, the extent of species-rich semi-natural grasslands has been drastically declining throughout Europe. Until the mid-20th century (and more recently in some areas) European grassland

agriculture was generally of low intensity, enabling habitat diversity to co-exist with food production (Pärtel *et al.*, 2005). In the decades since the 1940s there has been a drastic decline in the extent and connectivity of semi-natural grasslands as a consequence of the intensification of agriculture (Emanuelsson 2006). The remaining grasslands are fragmented and scattered within the landscape and the cessation of former management practices has led to overgrowth of many localities by shrubs and trees (Lipský, 1995; Poschlod & Bonn, 1998; Eriksson *et al.*, 2002; Luoto *et al.*, 2003).

Extensive research has been carried out in European grasslands in last decades, to explain origins of diversity and to provide guidelines for its conservation: It was shown that existence of species rich grasslands is strongly determined by habitat conditions, especially soil nutrients (Janssens *et al.*, 1998; Lobel *et al.*, 2006; Karlík & Poschlod, 2009). Some of these factors are influenced climatically or geologically, but inherent prerequisite of grasslands diversity is proper management such as mowing or grazing which often reduce dominance of few competitive species (Olff & Ritchie, 1998; Jones & Hayes, 1999; Hofmann & Isselstein, 2004; Pärtel *et al.*, 2005; Pywell *et al.*, 2007). Land use history of individual sites can thus have strong effect on present species richness and species composition (Gustavsson *et al.*, 2007; Chýlová & Münzbergová, 2008; Cousins *et al.*, 2009; Karlík & Poschlod, 2009).

With the ongoing habitat loss and fragmentation, it becomes even more important to identify factors affecting occurrence of species to ensure effective conservation of diversity at landscape scales. Importance of broader spatial context for individual populations or communities was first recognized in animals and it has been studied in plants not until 1990's. By that time, explanations of species distributions have often been based on local environmental conditions and local biotic interactions or on patterns over large geographical scales (Dupré & Ehrlén, 2002). In contrast, metapopulation theory (Hanski, 1994, 1999) stresses the importance of habitat configuration at an intermediate scale, in terms of area and isolation of habitat patches. Habitat isolation is known to be an important factor reducing the species richness of dispersal-limited taxa in a wide range of habitats. For grassland plants, however, the effect of present habitat isolation on species richness is often thought to be obscured by land use history (Lindborg & Eriksson, 2004; Helm *et al.*, 2006; Gustavsson *et al.*, 2007). Even highly isolated grasslands might be species rich due to the longevity and persistence of species from a time when the grassland was larger and/or more connected to other grasslands (Eriksson *et al.*, 2002). Nonetheless, some authors indeed found that less isolated grasslands are richer in species than more isolated ones (Bruun, 2000; Adriaens *et al.*, 2006).

Discrepancy between results of different authors on the effects of habitat isolation might be to large extent given by inconsistent methodology. There is a question what can be considered to be source or suitable habitat (Tremlová & Münzbergová, 2007). Further, what will be the measure or weight of habitat importance? Often, authors use area as a proxy of habitat importance as a source of propagules (Dupré & Ehrlén, 2002). The use of area in assessing isolation is based on the assumption that larger patches exhibit higher species richness and host larger populations and, thus, may provide more possible colonizers for the target patch (Hanski, 1999; Kiviniemi, 2008). In fragmented grasslands, however, a number of studies have failed to reveal a positive relationship between patch area and species richness (Eriksson *et al.*, 1995; Kiviniemi & Eriksson, 1999; Pärtel & Zobel, 1999) or patch area and population size (Eriksson & Ehrlén, 2001; Bruun, 2005). This may be caused by different habitat conditions of the source patches resulting from factors such as different land-use histories at these patches (Lindborg *et al.*, 2005; Chýlová & Münzbergová, 2008). There are also numerous ways how to calculate habitat isolation metrics (Moilanen & Nieminen, 2002; Prugh, 2009). And last, effect of isolation could be tested separately (Lindborg & Eriksson, 2004; Öster *et al.*,

2007; Bruckmann *et al.*, 2010) or together with other habitat characteristics such as abiotic factors (Adriaens *et al.*, 2006; Lobel *et al.*, 2006). Given all these above mentioned (and possibly also few others) factors, our understanding of the role of habitat isolation in grasslands is still limited because individual studies are hardly comparable.

A particular habitat configuration may be "perceived" differently by species with different sets of traits, and inter-patch distances that are easily overcome by species with good dispersal may constitute dispersal barriers to others (Dupré & Ehrlén, 2002). As a result, some species are much more susceptible to changes in landscape structure (Tremlová & Münzbergová, 2007). Nonetheless, high seed production or high abundance in the landscape might compensate for otherwise low dispersal ability. In grassland (and also other) plants, however, dispersal limitation is most directly assessed using seed addition experiments (Tilman, 1997; Münzbergová, 2004; Zeiter *et al.*, 2006; Öster *et al.*, 2009b; Seabloom, 2011). These experiments can not distinguish whether the failure of a species to colonize suitable habitat was due to its dispersal traits or due to low availability of seeds in the surrounding landscape. Such knowledge, however, is an essential baseline for any further conservation efforts (Murray *et al.*, 2002; Heywood & Iriondo, 2003).

In recent decades, socio-economic changes and new technologies employed in agriculture have brought about the abandonment of arable fields that were no longer profitable to maintain (Cramer *et al.*, 2008). Abandoned fields represent open, treeless habitats, and they are hence perceived as alternative habitats for species from declining grasslands (Walker et al., 2004). Indeed, many species are able to spontaneously colonise abandoned fields (Ruprecht, 2006; Öster *et al.*, 2009a; Knappová *et al.*, 2012). However, many other species are absent from communities that develop in abandoned fields and an important question remains what limits their successful establishment (Cramer *et al.*, 2008; Fagan *et al.*, 2008; Knappová *et al.*, 2012). Moreover, by answering this question, we can gain also important insights into factors determining species distribution in grasslands because colonization of recently abandoned fields by grassland species is the ongoing process which is not obscured by historical changes in landscape structure.

Study system and basic aims

Dry grasslands which first motivated this thesis occur in small fragments in the northern part of the Czech Republic and they host a vast small-scale diversity of vascular plants (Münzbergová, 2004; Chýlová & Münzbergová, 2008), including a number of threatened species. At present, most of the grasslands in the region are not managed, and occasionally, some of them are completely destroyed by human activities (e.g., plowing or development of solar power plants). A previous study in the same region demonstrated some portions of the current area of dry grasslands were arable fields in the 1950's or even in 1980's (Chýlová & Münzbergová, 2008), suggesting that grassland species have been able to spread into novel habitats. In the past, fields, orchards, pastures, vineyards and grasslands formed a small-grain heterogeneous mosaic. Therefore, a mixture of different land use histories can be found containing both continuous grasslands and relatively recently (e.g., in the 1980s) abandoned fields within a single current grassland (Chýlová & Münzbergová, 2008).

However, due to changes in agriculture, the fields in the current landscape are much larger and farther from the source grasslands than they were in the past. Moreover, increased application of fertilizers and the use of deep cultivation in the last decades might have considerably altered soil conditions. Abandoned fields also represent dynamic habitat with rapid vegetation changes (Osbornová *et al.*, 1990; Bartha *et al.*, 2003). Vegetation canopy might either compete with emerging seedlings or facilitate their establishment and it could differently affect seedlings and adult individuals.

Moreover, the effects of vegetation might change with spatiotemporally variable factors such as weather or soil conditions. I therefore assume that species' colonization of currently abandoned fields will be limited both by habitat suitability and species' dispersal ability as well as by resident vegetation of the fields. Differences among species in colonization success regarding different factors should be also reflected in differences in their traits.

The very basic aim of this thesis was to evaluate the *status quo* of dry grassland plants in abandoned fields. How many of so called grassland specialists were able to colonize fields abandoned in last two decades (Chapter 1). And in the second step, to identify what are the main constraints of successful colonization, both at the level of environment and of species traits. At the landscape level, I wonder what is the effect of isolation of an abandoned field from source dry grasslands on number of grassland species colonizing the field and how does the detected effect of isolation change with the method used for its calculation (Chapter 1). To determine the ability of a range of dry grassland species to really become established in abandoned fields, I performed seed addition experiment (Chapter 2). I experimentally manipulated resident vegetation to assess its general effect on species establishment, growth and reproduction. In addition, I asked to what extent the ability of species to establish in abandoned field is limited by species traits and habitat conditions, and whether this can be influenced by resident vegetation at these sites.

Subsequently, I used information assembled in a field survey (Chapter 1), seed sowing experiment (Chapter 2) and gathered from unpublished data and databases to assess whether successful establishment of dry grassland species in the studied abandoned fields is limited by habitat requirements of species, species traits related to dispersal or by availability of seeds in the landscape. In particular, I was interested in the relative importance of the three above mentioned sources of limitation for species occurrences on abandoned fields (Chapter 3). Finally, I went more in detail to the level of individual fields to see whether there is difference between field edges and interiors in species richness and species composition of grassland plants and whether differences in species colonization ability (expressed as species affinity to field edges) can be attributed to differences in dispersal traits, habitat requirements or seed availability (Chapter 4).

Main results

I revealed an unexpectedly high success of grassland species in the colonization of fields abandoned in the last two decades (Chapter 1). These abandoned fields may thus play an important role in the landscape dynamics of many grassland species, but their dispersal is probably often limited to short distances (less than 0.5 km). The proximity of species-rich rather than large source habitats was shown to be important for field colonization.

Results partly changed with the way how isolation measure was calculated, but our new isolation measure using the distance and species richness of surrounding habitats always explain more variability in species richness than does commonly used isolation measure based on distance end area of surrounding habitats. The different performance of the two isolation measures can be explained by the weak species–area relationship in the grasslands, indicating differences in their habitat quality. Species richness is a better proxy of habitat importance in terms of propagule source than habitat area, and the new isolation measure is therefore suitable for studying the effects of landscape structure on species richness in landscapes presenting a weak species–area relationship, such as areas exhibiting pronounced effects of land-use history. I have also shown that inclusion of habitat characteristics as covariates may considerably alter conclusions about the effects

of isolation, which could actually be overestimated when assessed separately. I therefore highly recommend considering habitat characteristics when studying habitat isolation. Through comparison of results obtained with and without inclusion of habitat characteristics, we may gain novel insights into factors affecting species richness.

In the seed addition experiment (Chapter 2), almost one half of the 35 sown species reproduced within two years after sowing, while two species completely failed to become established. The vegetation in the undisturbed plots facilitated seedling establishment only in the year with low spring precipitation, and the effect did not hold for all species. In contrast, growth and reproduction were consistently much greater in the disturbed plots, but the effect size depended on soil properties of each field. In accord with findings of field survey, abandoned fields really provide suitable habitats for a number of grassland species, but the probability of establishing a viable and fertile population is largely constrained by the vigour of resident vegetation. However, none of the investigated plant traits significantly explained species-specific response to disturbance, neither in proportion of established individuals, nor in plant size.

Seasonal variability involved in our study emphasizes the necessity of temporal replication of sowing experiments. Our results also highlight the importance of following the whole plant life cycle when assessing habitat suitability. Although this point has been stressed by some authors (Turnbull *et al.*, 2000), it has been overlooked even in recent studies (e.g., Öster et al., 2009a). Regarding the effect of resident vegetation on seedling establishment and growth, studies assessing habitat suitability should either involve both vegetation removal treatments and untreated, control plots (e.g., Vítová & Lepš, 2011), or deliberately cover the widest range of canopy density within the studied habitat. Such an approach could provide novel insights into factors limiting species distribution.

In Chapter 3, I showed that many dry grassland species fail to reach potentially suitable abandoned fields not because of their poor dispersal traits but due to low availability of seeds in the surrounding landscape. The latter is given mainly by number of source populations and only slightly by seed production and length of flowering period. I did not find much the same evidence in current literature though almost no studies differentiating the two sources of dispersal limitation exist. Our results thus bring important finding that the role of dispersal *per se* might not be the main cause of dispersal limitation detected by amounts of seed addition experiments.

The tight relationship between species frequency in dry grasslands and in abandoned fields also implies some kind of positive feedback. Once a species become frequent in source habitats, it is also much likely to be frequent in target habitats, more than expected from its trait-driven colonization ability. Such positive feedback does not need to apply only in case of colonization of novel habitats, but likely also on distribution patterns in existing grasslands. Being solely a result of neutral mechanisms, rare species are more extinction-prone, and once they go locally extinct, they take longer to re-immigrate than do common species (Volkov *et al.*, 2003). Our results thus provide important empirical support that both neutral and niche processes are taking part in assembly of ecological communities.

I confirmed the importance of seed availability (frequency in source grasslands) also at finer scale (Chapter 4). Field interiors were undersaturated with target dry grassland species compared to field edges. However, some species occur almost evenly in interiors as well as in edges, whereas others were found mostly in edges. I expected that difference among species is given either by their ability to disperse (poor dispersers would occur predominantly in edges) or by their habitat requirements (abandoned fields are rather unsuitable habitat for species occurring mainly in the edges and their populations here are maintained by seed supply from nearby sources). However, neither traits related to dispersal nor habitat requirements explained differences among species in

their affinity to field edges. It is especially striking that although edges were found to be slightly warmer and drier than interiors (Appendix 2), they were not preferably colonized by species with higher temperature or low moisture demands. It is possible that rather than favouring species according to their habitat requirements, warmer and drier conditions make resident vegetation less dense providing more microsites for establishment. Therefore, compare to interiors edges could be colonized also by species with low seed pressure, which are unable to reach scarce microsites in denser field interiors. Indeed, species that occur almost evenly in edges and interiors are species frequent in dry grasslands within the study area whereas species restricted to field edges occur sporadically in dry grasslands.

I found pronounced effect of source quality on species richness and species composition of grassland plants in field edges. The effect of source quality on species richness, however, diminished with distance from edge into interiors. This suggests that large fraction of species colonizing preferably field edges also depends on neighbourhood of high quality habitat whereas species that often colonize even interiors mainly occur independently on source habitat quality. Species with high light demands and low nutrient, moisture and soil reaction demands were more restricted to field edges neighbouring with habitat of high quality. All these traits characterize grassland specialists, which are unlikely to occur in low quality source habitats. Although these species seem to be dispersal limited I did not find any significant difference in species traits. Possible explanation is that lack of seeds resulting in colonization failure is not given primarily by dispersal *per se* (represented by dispersal traits), but to seed availability in landscape context (i.e., number of source populations or seed production in these populations). This explanation is in agreement with above discussed findings that species affinity to edges is related to traits species frequency in source grasslands and also with Chapter 3.

Conclusion and future directions

I have successfully tested novel approach for measuring habitat isolation, using distance and species richness of source habitats (Chapter 1). Assessing the species richness of source habitats would appear to be much more time-consuming than just calculating their areas. However, in the majority of studies addressing habitat isolation, there is no distinction made between target and source habitats, and species richness is known for all habitats under study to be the main response variable. Therefore, there is no additional effort needed for this type of assessment (except for substitution into a formula), and our approach might be easily applied and further tested. I have also shown that inclusion of habitat characteristics when testing for the effect of habitat isolation might considerably alter conclusions. I therefore recommend using both tests with and without inclusion of habitat characteristics in the future. I believe that these methodological improvements might push forward our understanding of factors that influence species richness not only in grasslands but also in other communities of perennial plants restricted to fragmented habitats.

I proved that abandoned fields in the study region could be successfully colonized by species that are also common in dry grasslands. For these species, abandoned fields represent suitable habitat. However, existence of abandoned fields might be only transient, depending on decisions of landowners and land managers. Nevertheless, even if an abandoned field supports a population of a species for only a few years, such transient population might still positively influence species landscape dynamics and the persistence of the species at the landscape scale (Loehle, 2007). However, rare dry grassland species occur only sporadically in these fields and mainly in edges only. Our

results indirectly indicate that low microsite availability together with low seed pressure is most limiting for these species. They are therefore much more restricted to dry grasslands and their conservation mostly relies on proper management of their current habitats.

In general, I found that species colonization success can be only hardly predicted based on traits related to dispersal, habitat requirements or competitive ability. The best predictor I found was niche width (Chapter 3), which also partly correlated with species frequency in source grasslands. The latter was by far the most strongly related to species frequency in abandoned fields (Chapter 3) and also to species ability to equally colonize field edges and interiors (Chapter 4). Although I could certainly miss some important traits (e.g. mycorrhizal dependence, see below), I think that it is unlikely that despite a wide range of traits under study there are still some very influential and phylogenetically independent hidden traits that determine species abundance in both source and target habitats. Rather, this tight relationship between species frequency in dry grasslands and in abandoned fields implies some kind of positive feedback and indirectly confirms the role of neutral mechanisms in community assembly (Volkov *et al.*, 2003).

I have considered a wide range of factors, that can influence successful colonization of abandoned fields by grassland plants, but other possibilities still remain unexplored. For example, there is increasing evidence that many plant species including grassland specialists are dependent on mycorrhizal symbiosis and that character of soil biota (abundance and species composition) might to large extent influence structure of plant communities (van der Heijden *et al.*, 1998; Hartnett & Wilson, 1999, 2002). At the same time, mycorrhizal fungi are largely influenced by soil conditions and therefore also by land use management, such as tillage or application of fertilizers (Eriksson, 2001; Jansa *et al.*, 2002; Oehl *et al.*, 2005). Changes in communities of soil symbionts caused by previous management of the fields might therefore alter conditions for grassland species and hinder their successful establishment. This alternative, however, need to be further tested and I am currently involved in running experiment based on this hypothesis.

There are also topics concerning dry grasslands alone that remain obscured. Although we know a lot about land-use history in individual grassland fragments and about habitat conditions that influence species richness and species composition, a large portion of variability still remains unexplained. I realised that individual grasslands are often dominated by one out of four species (*Anthericum ramosum, Brachypodium pinnatum, Bromus erectus* or *Inula salicina*). Though identity of dominant species might considerably influence community composition (Emery & Gross, 2006, 2007), we can gain much understanding from detecting origins of species dominance. It can be determined by habitat conditions but also by priority effects and subsequent positive feedbacks between species and its environment (Körner *et al.*, 2008; Fukami & Nakajima, 2011; van de Voorde *et al.*, 2011). Currently I am running both garden and field experiments (in cooperation with colleague Lucie Hemrová) to get answers on these questions.

To sum up, my thesis has described in detail current state of abandoned fields in study region and evaluated success of dry grassland species in colonization of these fields. I have assessed number of factors that influence colonization process, both from environmental and species perspective. Nevertheless, the study system of dry grasslands and abandoned fields still offer challenges for future research and provide opportunities for testing interesting ecological hypotheses.

References

Adriaens, D., Honnay, O., & Hermy, M. (2006) No evidence of a plant extinction debt in highly fragmented calcareous grasslands in Belgium. *Biological Conservation*, **133**, 212-224.

Aguiar, M.R. & Sala, O.E. (1994) Competition, Facilitation, Seed Distribution and the Origin of Patches in a Patagonian Steppe. *Oikos*, **70**, 26-34.

Bartha, S., Meiners, S.J., Pickett, S.T.A., & Cadenasso, M.L. (2003) Plant colonization windows in a mesic old field succession. *Applied Vegetation Science*, **6**, 205-212.

Bruckmann, S.V., Krauss, J., & Steffan-Dewenter, I. (2010) Butterfly and plant specialists suffer from reduced connectivity in fragmented landscapes. *Journal of Applied Ecology*, **47**, 799-809.

Bruun, H.H. (2000) Patterns of species richness in dry grassland patches in an agricultural landscape. *Ecography*, **23**, 641-650.

Bruun, H.H. (2005) A field test of the relationship between habitat area and population size for five perennial plant species. *Web ecology*, **5**, 1-5.

Callaway, R.M. & Walker, L.R. (1997) Competition and facilitation: A synthetic approach to interactions in plant communities. *Ecology*, **78**, 1958-1965.

Cousins, S.A.O., Lindborg, R., & Mattsson, S. (2009) Land use history and site location are more important for grassland species richness than local soil properties. *Nordic Journal of Botany*, **27**, 483-489.

Cramer, V.A., Hobbs, R.J., & Standish, R.J. (2008) What's new about old fileds? Land abandonment and ecosystem assembly. *Trends in Ecology & Evolution*, **23**, 104-112.

Diaz, S., Cabido, M., & Casanoves, F. (1998) Plant functional traits and environmental filters at a regional scale. *Journal of Vegetation Science*, **9**, 113-122.

Dupré, C. & Ehrlén, J. (2002) Habitat configuration, species traits and plant distributions. *Journal of Ecology*, **90**, 796-805.

Emanuelsson, U. (2008) Semi-natural grasslands in Europe today. *Grassland Science in Europe*, **13**, 3-8.

Emery, S.M. & Gross, K.L. (2006) Dominant species identity regulates invasibility of old-field plant communities. *Oikos*, **115**, 549-558.

Emery, S.M. & Gross, K.L. (2007) Dominant species identity, not community evenness, regulates invasion in experimental grassland plant communities. *Ecology*, **88**, 954-964.

Eriksson, A. (2001) Arbuscular mycorrhiza in relation to management history, soil nutrients and plant species diversity. *Plant Ecology*, **155**, 129-137.

Eriksson, A., Eriksson, O., & Berglund, H. (1995) Species Abundance Patterns of Plants in Swedish Seminatural Pastures. *Ecography*, **18**, 310-317.

Eriksson, O., Cousins, S.A.O., & Bruun, H.H. (2002) Land-use history and fragmentation of traditionally managed grasslands in Scandinavia. *Journal of Vegetation Science*, **13**, 743-748.

Eriksson, O. & Ehrlén, J. (2001). Landscape fragmentation and the viability of plant populations. In *Integrating ecology and evolution in spatial context* (eds J. Silwertown & J. Antonovics), pp. 157-175. Blackwell, Oxford.

Fagan, K.C., Pywell, R.F., Bullock, J.M., & Marrs, R.H. (2008) Do restored calcareous grasslands on former arable fields resemble ancient targets? The effect of time, methods and environment on outcomes. *Journal of Applied Ecology*, **45**, 1293-1303.

Farrer, E.C. & Goldberg, D.E. (2011) Patterns and mechanisms of conspecific and heterospecific interactions in a dry perennial grassland. *Journal of Ecology*, **99**, 265-276.

Fukami, T. & Nakajima, M. (2011) Community assembly: alternative stable states or alternative transient states? *Ecology Letters*, **14**, 973-984.

Ganade, G. & Brown, V.K. (2002) Succession in old pastures of central Amazonia: Role of soil fertility and plant litter. *Ecology*, **83**, 743-754.

Gravel, D., Canham, C.D., Beaudet, M., & Messier, C. (2006) Reconciling niche and neutrality: the continuum hypothesis. *Ecology Letters*, **9**, 399-409.

Gustavsson, E., Lennartsson, T., & Emanuelsson, M. (2007) Land use more than 200 years ago explains current grassland plant diversity in a Swedish agricultural landscape. *Biological Conservation*, **138**, 47-59.

Hanski, I. (1994) A Practical Model of Metapopulation Dynamics. *Journal of Animal Ecology*, **63**, 151-162.

Hanski, I. (1999) Habitat connectivity, habitat continuity, and metapopulations in dynamic landscapes. *Oikos*, **87**, 209-219.

Hartnett, D.C. & Wilson, G.W.T. (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, **80**, 1187-1195.

Hartnett, D.C. & Wilson, G.W.T. (2002) The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. *Plant and Soil*, **244**, 319-331.

Helm, A., Hanski, I., & Partel, M. (2006) Slow response of plant species richness to habitat loss and fragmentation. *Ecology Letters*, **9**, 72-77.
Heywood, V.H. & Iriondo, J.M. (2003) Plant conservation: old problems, new perspectives. *Biological Conservation*, **113**, 321-335.
Hofmann, M. & Isselstein, J. (2004) Seedling recruitment on agriculturally improved mesic grassland: the influence of disturbance and management schemes. *Applied Vegetation Science*, **7**, 193-200.
Holmgren, M. & Scheffer, M. (2010) Strong facilitation in mild environments: the stress gradient hypothesis revisited. *Journal of Ecology*, **98**, 1269-1275.
Hopkins, A. (2009) Grasses and grassland ecology. *Grass and Forage Science*, **64**, 339-339.
Hopkins, A. & Holz, B. (2005). Grassland for agriculture and nature conservation: production, quality and multi-functionality. In *Integrating Efficient Grassland Farming and Biodiversity*, Vol. 10, pp. 15-29.
Hubbell, S.P. (2001) *The unified neutral theory of biodiversity and biogeography* Princeton University Press, Princeton, N.J. ; Oxford.
Choler, P., Michalet, R., & Callaway, R.M. (2001) Facilitation and competition on gradients in alpine plant communities. *Ecology*, **82**, 3295-3308.
Chýlová, T. & Münzbergová, Z. (2008) Past land use co-determines the present distribution of dry grassland plant species. *Preslia*, **80**, 183-198.
Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I.R., & Frossard, E. (2002) Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza*, **12**, 225-234.
Janssens, F., Peeters, A., Tallowin, J.R.B., Bakker, J.P., Bekker, R.M., Fillat, F., & Oomes, M.J.M. (1998) Relationship between soil chemical factors and grassland diversity. *Plant and Soil*, **202**, 69-78.
Jones, A.T. & Hayes, M.J. (1999) Increasing floristic diversity in grassland: the effects of management regime and provenance on species introduction. *Biological Conservation*, **87**, 381-390.
Jones, C.G., Lawton, J.H., & Shachak, M. (1997) Positive and negative effects of organisms as physical ecosystem engineers. *Ecology*, **78**, 1946-1957.
Karlík, P. & Poschlod, P. (2009) History or abiotic filter: which is more important in determining the species composition of calcareous grasslands? *Preslia*, **81**, 321-340.
Kiviniemi, K. (2008) Effects of fragment size and isolation on the occurrence of four short-lived plants in semi-natural grasslands. *Acta Oecologica-International Journal of Ecology*, **33**, 56-65.
Kiviniemi, K. & Eriksson, O. (1999) Dispersal, recruitment and site occupancy of grassland plants in fragmented habitats. *Oikos*, **86**, 241-253.
Knappová, J., Hemrová, L., & Münzbergová, Z. (2012) Colonization of central European abandoned fields by dry grassland species depends on the species richness of the source habitats: a new approach for measuring habitat isolation. *Landscape Ecology*, **27**, 97-108.
Körner, C., Stocklin, J., Reuther-Thiebaud, L., & Pelaez-Riedl, S. (2008) Small differences in arrival time influence composition and productivity of plant communities. *New Phytologist*, **177**, 698-705.
Kraft, N.J.B., Valencia, R., & Ackerly, D.D. (2008) Functional traits and niche-based tree community assembly in an amazonian forest. *Science*, **322**, 580-582.
Kylafis, G. & Loreau, M. (2011) Niche construction in the light of niche theory. *Ecology Letters*, **14**, 82-90.
Lindborg, R., Cousins, S.A.O., & Eriksson, O. (2005) Plant species response to land use change - Campanula rotundifolia, Primula veris and Rhinanthus minor. *Ecography*, **28**, 29-36.
Lindborg, R. & Eriksson, O. (2004) Historical landscape connectivity affects present plant species diversity. *Ecology*, **85**, 1840-1845.
Lipský, Z. (1995) The Changing Face of the Czech Rural Landscape. *Landscape and Urban Planning*, **31**, 39-45.
Lobel, S., Dengler, J., & Hobohm, C. (2006) Species richness of vascular plants, bryophytes and lichens in dry grasslands: The effects of environment, landscape structure and competition. *Folia Geobotanica*, **41**, 377-393.
Loehle, C. (2007) Effect of ephemeral stepping stones on metapopulations on fragmented landscapes. *Ecological Complexity*, **4**, 42-47.
Luoto, M., Rekolainen, S., Aakkula, J., & Pykala, J. (2003) Loss of plant species richness and habitat connectivity in grasslands associated with agricultural change in Finland. *Ambio*, **32**, 447-452.
McGill, B.J., Enquist, B.J., Weiher, E., & Westoby, M. (2006) Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, **21**, 178-185.
Moilanen, A. & Nieminen, M. (2002) Simple connectivity measures in spatial ecology. *Ecology*, **83**, 1131-1145.
Münzbergová, Z. (2004) Effect of spatial scale on factors limiting species distributions in dry grassland fragments. *Journal of Ecology*, **92**, 854-867.
Münzbergová, Z. & Herben, T. (2005) Seed, dispersal, microsite, habitat and recruitment limitation: identification of terms and concepts in studies of limitations. *Oecologia*, **145**, 1-8.

Murray, B.R., Thrall, P.H., Gill, A.M., & Nicotra, A.B. (2002) How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecology*, **27**, 291-310.
Myers, J.A. & Harms, K.E. (2009) Seed arrival, ecological filters, and plant species richness: a meta-analysis. *Ecology Letters*, **12**, 1250-1260.
Oehl, F., Sieverding, E., Ineichen, K., Ris, E.A., Boller, T., & Wiemken, A. (2005) Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. *New Phytologist*, **165**, 273-283.
Olff, H. & Ritchie, M.E. (1998) Effects of herbivores on grassland plant diversity. *Trends in Ecology & Evolution*, **13**, 261-265.
Osbornová, J., Kovářová, M., Lepš, J., Prach, K., & [eds.] (1990) *Succession in abandoned fields: studies in central Bohemia, Czechoslovakia* Kluwer Academic, Dordrecht ; London.
Öster, M., Ask, K., Cousins, S.A.O., & Eriksson, O. (2009a) Dispersal and establishment limitation reduces the potential for successful restoration of semi-natural grassland communities on former arable fields. *Journal of Applied Ecology*, **46**, 1266-1274.
Öster, M., Ask, K., Römermann, C., Tackenberg, O., & Eriksson, O. (2009b) Plant colonization of ex-arable fields from adjacent species-rich grasslands: The importance of dispersal vs. recruitment ability. *Agriculture Ecosystems & Environment*, **130**, 93-99.
Öster, M., Cousins, S.A.O., & Eriksson, O. (2007) Size and heterogeneity rather than landscape context determine plant species richness in semi-natural grasslands. *Journal of Vegetation Science*, **18**, 859-868.
Ozinga, W.A., Schaminee, J.H.J., Bekker, R.M., Bonn, S., Poschlod, P., Tackenberg, O., Bakker, J., & van Groenendael, J.M. (2005) Predictability of plant species composition from environmental conditions is constrained by dispersal limitation. *Oikos*, **108**, 555-561.
Pärtel, M., Bruun, H.H., & Sammul, M. (2005). Biodiversity in temperate European grasslands: origin and conservation. In *Integrating Efficient Grassland Farming and Biodiversity*, Vol. 10, pp. 1-14.
Pärtel, M. & Zobel, M. (1999) Small-scale plant species richness in calcareous grasslands determined by the species pool, community age and shoot density. *Ecography*, **22**, 153-159.
Poschlod, P. & Bonn, S. (1998) Changing dispersal processes in the central European landscape since the last ice age: an explanation for the actual decrease of plant species richness in different habitats? *Acta Botanica Neerlandica*, **47**, 27-44.
Prugh, L.R. (2009) An evaluation of patch connectivity measures. *Ecological Applications*, **19**, 1300-1310.
Pywell, R.F., Bullock, J.M., Tallowin, J.B., Walker, K.J., Warman, E.A., & Masters, G. (2007) Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints. *Journal of Applied Ecology*, **44**, 81-94.
Ruprecht, E. (2006) Successfully recovered grassland: A promising example from Romanian old-fields. *Restoration Ecology*, **14**, 473-480.
Seabloom, E.W. (2011) Spatial and temporal variability in propagule limitation of California native grasses. *Oikos*, **120**, 291-301.
Shipley, B., Vile, D., & Garnier, E. (2006) From plant traits to plant communities: A statistical mechanistic approach to biodiversity. *Science*, **314**, 812-814.
Schleicher, A., Biedermann, R., & Kleyer, M. (2011) Dispersal traits determine plant response to habitat connectivity in an urban landscape. *Landscape Ecology*, **26**, 529-540.
Tilman, D. (1997) Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology*, **78**, 81-92.
Tilman, D. (2004) Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 10854-10861.
Tremlová, K. & Münzbergová, Z. (2007) Importance of species traits for species distribution in fragmented landscapes. *Ecology*, **88**, 965-977.
Turnbull, L.A., Crawley, M.J., & Rees, M. (2000) Are plant populations seed-limited? A review of seed sowing experiments. *Oikos*, **88**, 225-238.
van de Voorde, T.F.J., van der Putten, W.H., & Bezemer, T.M. (2011) Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession. *Journal of Ecology*, **99**, 945-953.
van de Voorde, T.F.J., van der Putten, W.H., & Bezemer, T.M. (2012) Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession. *Journal of Ecology*, **99**, 945-953.
van der Heijden, M.G.A., Boller, T., Wiemken, A., & Sanders, I.R. (1998) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology*, **79**, 2082-2091.
Veblen, K.E. (2008) Season- and herbivore-dependent competition and facilitation in a semiarid savanna. *Ecology*, **89**, 1532-1540.

Violle, C., Garnier, E., Lecoeur, J., Roumet, C., Podeur, C., Blanchard, A., & Navas, M.L. (2009) Competition, traits and resource depletion in plant communities. *Oecologia*, **160**, 747-755.
Vítová, A. & Lepš, J. (2011) Experimental assessment of dispersal and habitat limitation in an oligotrophic wet meadow. *Plant Ecology*, **212**, 1231-1242.
Vlasáková, B., Raabová, J., Kyncl, T., Dostál, P., Kovářová, M., Kovář, P., & Herben, T. (2009) Ants accelerate succession from mountain grassland towards spruce forest. *Journal of Vegetation Science*, **20**, 577-587.
Volkov, I., Banavar, J.R., Hubbell, S.P., & Maritan, A. (2003) Neutral theory and relative species abundance in ecology. *Nature*, **424**, 1035-1037.
Walker, K.J., Stevens, P.A., Stevens, D.P., Mountford, J.O., Manchester, S.J., & Pywell, R.F. (2004) The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation*, **119**, 1-18.
Walker, L.R., Bellingham, P.J., & Peltzer, D.A. (2006) Plant characteristics are poor predictors of microsite colonization during the first two years of primary succession. *Journal of Vegetation Science*, **17**, 397-406.
Zeiter, M., Stampfli, A., & Newbery, D.M. (2006) Recruitment limitation constrains local species richness and productivity in dry grassland. *Ecology*, **87**, 942-951.

Colonization of central European abandoned fields by dry grassland species depends on the species richness of the source habitats: a new approach for measuring habitat isolation

Jana Knappová, Lucie Hemrová & Zuzana Münzbergová (2012)

Landscape Ecol
DOI 10.1007/s10980-011-9680-5

RESEARCH ARTICLE

Colonization of central European abandoned fields by dry grassland species depends on the species richness of the source habitats: a new approach for measuring habitat isolation

Jana Knappová · Lucie Hemrová · Zuzana Münzbergová

Received: 4 February 2011 / Accepted: 3 November 2011

Abstract Abandoned fields are perceived as potential habitats for species of threatened semi-natural dry grasslands. However, information is lacking regarding how the spontaneous colonization of abandoned fields depends on the broader spatial context. We recorded the occurrence of 87 target species in 46 abandoned fields and 339 dry grasslands. We tested the effect of the isolation of abandoned fields from source grasslands on the number of dry grassland species occurring in abandoned fields either with or without habitat characteristics being used as covariates. The isolation of the fields was calculated using the distance and area (I_A) or distance and species richness (I_S) of source habitats. I_S always explained the number of grassland species in the abandoned fields better than I_A. The effect of isolation became smaller or even non-significant with the inclusion of covariates; it also changed with the method used for measuring distance (edge-to-edge or center-to-center), and it was lower when other abandoned fields were considered as additional source habitats. The different performance of the two isolation measures can be explained by the weak species–area relationship in the grasslands, indicating differences in their habitat quality. Species richness is a better proxy of habitat importance in terms of propagule source than habitat area, and the new isolation measure is therefore suitable for studying the effects of landscape structure on species richness in landscapes presenting a weak species–area relationship, such as areas exhibiting pronounced effects of land-use history. Inclusion of habitat characteristics as covariates may considerably alter conclusions regarding the effect of isolation, which might actually be overestimated when assessed separately.

Keywords Agricultural landscape · Connectivity · Czech Republic · Dispersal · Diversity · Fragmentation · *Festuco-Brometea* · Secondary succession

Electronic supplementary material The online version of this article (doi:10.1007/s10980-011-9680-5) contains supplementary material, which is available to authorized users.

J. Knappová
Department of Ecology, Faculty of Science, Charles University in Prague, Vinicna 7, 128 44 Prague, Czech Republic

J. Knappová · L. Hemrová · Z. Münzbergová
Department of Botany, Faculty of Science, Charles University in Prague, Benatska 2, 128 01 Prague, Czech Republic

J. Knappová (✉) · L. Hemrová · Z. Münzbergová
Institute of Botany, Academy of Sciences of the Czech Republic, Zamek 1, 252 43 Pruhonice, Czech Republic
e-mail: jana.knappova@ibot.cas.cz

Published online: 16 November 2011

Introduction

The extent of species-rich semi-natural grasslands has been drastically declining throughout Europe over the past century. The remaining grasslands are fragmented

and scattered within the landscape and the cessation of former management practices has led to overgrowth of many localities by shrubs and trees (Lipsky 1995; Poschlod and Bonn 1998; Eriksson et al. 2002; Luoto et al. 2003; Cousins 2009a). More recently, socio-economic changes and new technologies used in agriculture have brought about the abandonment of arable fields that were no longer profitable to maintain. This could be beneficial for grassland conservation, as the direct restoration of grasslands on ex-arable land is becoming a common practice (Pywell et al. 2002; Walker et al. 2004). Nevertheless, only a limited number of former fields can be intentionally converted into grasslands, and the question arises of whether fields can be successfully colonized by grassland species, even without conservation efforts (Ruprecht 2006; Oster et al. 2009a, b).

To assess the natural colonization of abandoned fields, the number of grassland species spontaneously growing in abandoned fields compared to the pool of species present in adjacent source grasslands must be known. However, the distribution of grassland plants in abandoned fields has only rarely been studied at a landscape scale (but see Ruprecht 2006; Cousins and Aggemyr 2008 for grazed ex-fields). Rather, studies addressing spatial patterns of species richness in abandoned fields usually only compare plots at different distances from an adjacent source habitat (Cook et al. 2005; Oster et al. 2009b).

The studies examining dispersal from a single source habitat focus on species' ability to disperse over relatively short distances, and dispersal from farther source habitats is overlooked. The broader spatial context of target fields can be quantified as the isolation of a field from source grasslands. Based on theory (Hanski 1999), the species richness of a particular habitat patch is expected to decrease with increasing isolation of this patch. However, many studies in grasslands have failed to demonstrate an effect of isolation on species richness (Lindborg and Eriksson 2004; Helm et al. 2006; Lobel et al. 2006; Oster et al. 2007). This may be caused by the very slow response of perennial plants to landscape changes (Helm et al. 2006; Cousins and Eriksson 2008; Cousins 2009b), indicating that species are in fact responding to landscape structure in the past and that their distribution is not in equilibrium with the current landscape (Lindborg and Eriksson 2004; Gustavsson et al. 2007). The ongoing process of colonization of recently abandoned fields by species from current grasslands provides an excellent opportunity to assess the effect of isolation on species richness without it being obscured by historical changes in landscape structure.

Recent work has shown that plant species richness varies in response to topography-related habitat characteristics, such as site-specific solar radiation and slope (Pykala et al. 2005; Bennie et al. 2006). The species richness of grassland plants is also related to soil conditions (Janssens et al. 1998). However, studies on the effect of isolation on the species richness of grassland plants usually do not include habitat characteristics as explanatory variables in models explaining species richness (e.g., Lindborg and Eriksson 2004; Oster et al. 2007; Bruckmann et al. 2010). Therefore, the resulting effect of habitat isolation on species richness might be in fact overestimated due to spatial correlation of habitat characteristics and isolation. We aim to compare the effect of isolation of abandoned fields from source grasslands with and without inclusion of habitat characteristics.

To describe habitat isolation, different studies use very different measures (Moilanen and Nieminen 2002; Kindlmann and Burel 2008; Prugh 2009). However, all measures that have been used thus far to study the effect of habitat isolation on species richness are based on the distance from possible sources and/or the size of the source habitat and do not consider the species richness at the sources. The use of area in assessing isolation is based on the assumption that larger patches exhibit higher species richness and host larger populations and, thus, may provide more possible colonizers for the target patch (Hanski 1999; Kiviniemi 2008). In fragmented grasslands, however, a number of studies have failed to reveal a positive relationship between patch area and species richness (Eriksson et al. 1995; Kiviniemi and Eriksson 1999; Partel and Zobel 1999) or patch area and population size (Eriksson and Ehrlen 2001; Bruun 2005). This may be caused by different habitat conditions of the source patches resulting from factors such as different land-use histories at these patches (e.g., Cousins 2001; Lindborg et al. 2005; Chylova and Munzbergova 2008). We therefore hypothesize that using the species richness of surrounding source habitats instead of area may provide more meaningful results than when calculating isolation based on area.

Our study area in northern Bohemia, Czech Republic is situated in a landscape associated with a

long tradition of agriculture and contains both grassland fragments and abandoned fields. This provides an excellent opportunity to examine and separate factors limiting the richness of grassland plants in abandoned fields. In this study, we performed an extensive field survey asking the following questions:

(i) How many dry grassland species were able to colonize arable fields abandoned in last two decades?
(ii) What is the effect of isolation of an abandoned field from source dry grasslands on number of grassland species colonizing the field?
(iii) How does the detected effect of isolation change with the method used for its calculation?
(iv) How does the detected effect of isolation change when habitat characteristics are included as covariates?

To answer these questions, we recorded the occurrence of 87 dry grassland plant species in 339 source dry grasslands and 46 target abandoned fields. For each abandoned field, we assessed a number of habitat characteristics that could be used as covariates. Finally, two different measures of the isolation of each field were calculated based either on area and distance or on species richness and the distance of surrounding source grasslands.

Methods

Study area and target species

The study area (8.5 by 8.5 km) is situated in the northern part of the Czech Republic (boundaries: 50°33′19.3″N, 14°14′25.1″E–50°33′47.4″N, 14°21′36.2″E; 50°28′46.3″N, 14°15′5.2″E–50°29′47.2″N, 14°21′40.9″E) at 200–270 m a.s.l. The long-term average temperature in the region is 7.7°C, and the long-term normal precipitation is 612 mm (Web 1). The prevailing bedrocks consist of sediments of different ages and origins, mainly sandstones and loess loams. The area is associated with a long tradition of agriculture with a prevalence crop production, accompanied by vineyards on steeper southern slopes and hop fields in alluvial areas. At present, agricultural land covers more than 70% of the study area (see map in Appendix 1 in Electronic supplementary material). Natural vegetation is represented by remnants of oak-hornbeam and thermophilous oak forests (alliance *Carpinion* and *Quercion petrae*, Ellenberg 1988), with total cover of approximately 10% within the study area. Calcareous dry grasslands (alliance *Bromion erecti*, Ellenberg 1988) occur in small fragments totaling 4% of the study area, and they host a vast small-scale diversity of vascular plants (Munzbergova 2004, Chylova and Munzbergova 2008), including a number of threatened species. At present, most of the grasslands in the region are not managed, and occasionally, some of them are completely destroyed by human activities (e.g., plowing or development of solar power plants). A previous study in the same region demonstrated some portions of the current area of dry grasslands were arable fields in the 1950s or even in 1980s (Chylova and Munzbergova 2008), suggesting that grassland species have been able to spread into novel habitats. In the past, fields, orchards, pastures, vineyards and grasslands formed a small-grain heterogeneous mosaic. Therefore, a mixture of different land use histories can be found containing both continuous grasslands and relatively recently (e.g., in the 1980s) abandoned fields within a single current grassland (Chylova and Munzbergova 2008). However, due to changes in agriculture, the fields in the current landscape are much larger and farther from the source grasslands than they were in the past. Moreover, increased application of fertilizers and the use of deep cultivation in the last decades might have considerably altered soil conditions. We therefore assume that species' colonization of currently abandoned fields will be limited both by habitat suitability and species' dispersal ability. Fields abandoned in last 20 years are already overgrown with grasses and ruderal herbaceous vegetation, e.g., *Arrhenatherum elatius*, *Dactylis glomerata*, *Cirsium arvense*, *Melilotus albus* and *M. officinalis*, and they make up approximately 1% of the study area.

Data collection

Field data collection

Based on studies performed within the same region (Munzbergova 2004; Tremlova and Munzbergova 2007; Chylova and Munzbergova 2008), we selected 87 target species as species restricted to dry grassland fragments (Table 1).

In 2009, using GPS, we located all fields abandoned in the last 15 years and all source grasslands within the

Table 1 List of target dry grassland species

Target species	Percentage occupied		Target species	Percentage occupied	
	Abandoned fields	Dry grasslands		Abandoned fields	Dry grasslands
Agrimonia eupatoria	96	92	*Koeleria pyramidata*	7	19
Coronilla varia	93	89	*Tanacetum corymbosum*	7	17
Fragaria viridis	89	81	*Asperula cynanchica*	7	15
Origanum vulgare	78	49	*Dianthus carthusianorum*	7	14
Inula salicina	76	67	*Veronica austriaca* subsp. *teucrium*	4	13
Festuca rupicola	74	75	*Aster amellus*	4	9
Knautia arvensis	72	88	*Melampyrum arvense*	4	9
Centaurea jacea	72	70	*Inula hirta*	4	3
Astragalus glycyphyllos	72	59	*Artemisia campestris*	4	2
Euphorbia cyparissias	67	81	*Centaurea rhenana*	4	2
Galium verum	67	73	*Thymus praecox*	2	27
Bupleurum falcatum	65	65	*Anthyllis vulneraria*	2	22
Brachypodium pinnatum	59	84	*Peucedanum cervaria*	2	16
Lotus corniculatus	57	78	*Genista tinctoria*	2	12
Salvia verticillata	54	50	*Potentilla arenaria*	2	11
Astragalus cicer	52	35	*Teucrium chamaedrys*	0	23
Centaurea scabiosa	48	65	*Helianthemum nummularium* subsp. *grandiflorum*	0	20
Trifolium medium	48	65	*Trifolium montanum*	0	18
Scabiosa ochroleuca	48	54	*Carex humilis*	0	17
Stachys recta	41	36	*Anemone sylvestris*	0	15
Plantago media	39	65	*Polygala vulgaris*	0	15
Linum catharticum	37	66	*Geranium sanguineum*	0	10
Sanguisorba minor	30	71	*Asperula tinctoria*	0	9
Carlina vulgaris	28	25	*Anthericum ramosum*	0	8
Eryngium campestre	26	46	*Melampyrum nemorosum*	0	7
Bromus erectus	22	40	*Sesleria albicans*	0	7
Gentiana cruciata	22	17	*Aster linosyris*	0	5
Cirsium eriophorum	22	9	*Filipendula vulgaris*	0	5
Hieracium pilosella	20	25	*Globularia elongata*	0	5
Pimpinella saxifraga	17	48	*Linum tenuifolium*	0	5
Medicago falcata	11	37	*Campanula rotundifolia*	0	4
Primula veris	11	31	*Listera ovata*	0	4
Ononis spinosa	11	22	*Linum flavum*	0	3
Euphrasia rostkoviana	11	3	*Onobrychis viciifolia*	0	3
Leontodon hispidus	9	46	*Scorzonera hispanica*	0	2
Briza media	9	44	*Seseli hippomarathrum*	0	2
Potentilla heptaphylla	9	42	*Thesium linophyllon*	0	2
Carex flacca	9	40	*Campanula glomerata*	0	1
Carex tomentosa	9	22	*Coronilla vaginalis*	0	1
Salvia nemorosa	9	13	*Gymnadenia conopsea*	0	1
Salvia pratensis	7	68	*Laserpitium latifolium*	0	1

Table 1 continued

Target species	Percentage occupied		Target species	Percentage occupied	
	Abandoned fields	Dry grasslands		Abandoned fields	Dry grasslands
Prunella vulgaris	7	32	*Melampyrum cristatum*	0	1
Cirsium acaule	7	29	*Scabiosa canescens*	0	1
Prunella grandiflora	7	29			

For each species, the percentages of occupied abandoned fields (from total 46) and dry grasslands (from total 339) are shown. The nomenclature follows Tutin et al. (1964–1983)

study area. A total of 46 abandoned fields ranging in size from 815 to 50,222 m^2 and 339 dry grasslands ranging in size from 6 to 274,800 m^2 were included. We defined a dry grassland as a site with visually homogenous vegetation separated from other localities by an unsuitable area and hosting at least one of the target dry grassland species. In cases of abrupt vegetation change within continuous grassland, the parts with different vegetation were treated as different localities. These cases were not common; in all of them there was a visual topographic barrier between the localities such as a small ditch or change of slope from very steep to flat.

In all of the abandoned fields and dry grasslands, we recorded the occurrence of each of 87 target species. During the field survey, we recorded only adult, usually flowering, individuals because small juvenile plants and seedlings are almost impossible to detect in the dense vegetation of abandoned fields. For the purpose of our study, the species found in the abandoned fields are referred to as *generalists.* Species only found in the dry grasslands are referred to as *specialists*. We use these terms for simplicity, mainly to separate the two groups of species, which could be also classified as *early* and *late colonizers* or *good* and *poor colonizers*.

Data on habitat characteristics

For each abandoned field, we determined several types of habitat characteristics (Table 2). Information on the time since the abandonment of each field (further referred to as "Age") was obtained through personal communication with landowners. As this information was only approximate, we divided the fields into three age categories: up to 7, 10 or 15 years. Several fields were also seeded with a commercial seed mixture in the last year before abandonment. The commercial seed mixture consisted of a few productive grasses and legumes (*Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Lolium multiflorum* and *Trifolium pratense*) and did not contain any of the target species. Information related to seeding is important because it may influence the establishment success of the target species at the study sites.

We used ArcGIS 9.2 (ESRI 2006) to obtain mean values for the TWI (topographic wetness index), Slope and potential direct solar irradiation (PDSI) from December to June for each abandoned field. Based on digital geological data, we also assessed the percentage cover of eight bedrock types in each abandoned field (further referred to as Geology, Table 2). See Appendix 2 in Electronic supplementary material for technical details of the GIS analyses and an extended description of habitat characteristics.

Isolation of abandoned fields

The majority of studies assessing the effect of isolation on plant species richness in grasslands use the isolation measure originating from the Incidence Function Model of metapopulation dynamics (Hanski 1994). To incorporate different distances of source patches, this measure uses a negative exponential dispersal kernel (the probability density function of the dispersal distance for an individual or population), with parameter α scaling the effect of distance to migration. However, parameter α is species specific and is difficult to accurately estimate in studies on multiple species. We therefore decided to use a simpler quadratic rational dispersal kernel (e.g., Tremlova and Munzbergova 2007).

First, we calculated the isolation of each abandoned field (I_A) as the mass of the surrounding source dry

Table 2 List of parameters collected for each abandoned field ($N = 46$) and their effect on the species richness of the abandoned fields

Parameter	df	Type		R^2
Coordinates				0.15
x	**1**	**Continual**		
y	**1**	**Continual**		
x*y	**1**	**Continual**		
Habitat characteristics				0.48
Age	1	Continual		
Seeding	**1**	**Binomial**	**−**	**0.05**
Area	**1**	**Log(continual)**	**+**	**0.05**
TWI	1	Continual		
Slope	**1**	**Continual**	**+**	**0.12**
PDSI_December	1	Continual		
PDSI_January	1	Continual		
PDSI_February	1	Continual		
PDSI_March	**1**	**Continual**	**−**	**<0.01**
PDSI_April	1	Continual		
PDSI_May	**1**	**Continual**	**+**	**0.01**
PDSI_June	**1**	**Continual**	**−**	**0.17**
Geology	**7**	**Factorial**		**0.08**

TWI topographic wetness index; *PDSI* potential direct solar irradiation

Parameters in bold were selected by step-wise regression and, thus, were included in the final model. The sign ± indicates a positive/negative relationship of the parameters in the model

grasslands weighted by their distance to the target abandoned field following Eq. 1.

$$I_{Aj} = -\log \sum_{k=1}^{n} \left[\left(A_k / d_{jk}^2 \right) \right], \quad j \neq k, \tag{1}$$

where I_j is the isolation of abandoned field j; k represents all of the surrounding grasslands within a 0.5 km distance of abandoned field j; A_k is the area of grassland k; and d_{jk} is the distance between abandoned field j and grassland k.

Second, we replaced the area of the surrounding grasslands with the number of target species occurring in each grassland and calculated isolation (I_S) following Eq. 2.

$$I_{Sj} = -\log \sum_{k=1}^{n} \left[\left(S_k / d_{jk}^2 \right) \right], \quad j \neq k, \tag{2}$$

where I_j is the isolation of abandoned field j; k represents all of the surrounding grasslands within a 0.5 km distance of abandoned field j; S_k is the number of target species in grassland k; and d_{jk} is the distance between abandoned field j and grassland k. We measured d_{jk} either as the center-to-center or edge-to-edge (shortest) distance.

The other surrounding fields could theoretically also serve as propagule sources for target species. Therefore, we considered surrounding abandoned fields as additional source habitats when calculating isolation and compared the results obtained from the models when abandoned fields were not considered.

The mean edge-to-edge distances between the source and target habitats were 253 and 226.4 m (range 1–500 m) when source habitats were represented either by grasslands or both grasslands and abandoned fields, respectively. The mean center-to-center distances were 288.9 and 271.5 m (range 20–500 m), respectively.

The resulting isolation value was higher for more isolated sites, i.e., abandoned fields that were farther from source grasslands and/or when the source grasslands were smaller (I_A) or hosted fewer target species (I_S). For the most isolated abandoned fields, $I_A = 1.88$, and $I_S = 3.83$. For the least isolated abandoned fields, $I_A = -5.12$, and $I_S = -2.01$.

Data analysis

To asses differences in the number of target species occurring in individual grasslands and abandoned fields, differences in the areas of the two habitat types must be taken into account. In a linear regression, the number of target species was employed as a dependent variable, and habitat type (grassland/field) and the logarithm of area and their interaction were used as independent variables. The relationship between the number of target species and the logarithm of habitat area was also analyzed separately for the two habitat types using linear regression. In grasslands, it was further tested separately for specialist and generalist species. Because some of the grasslands were either larger than the largest abandoned field or smaller than the smallest abandoned field, we also tested the species–area relationship using only grasslands within the same span of areas as the abandoned fields.

To identify factors determining the number of species occurring in the abandoned fields, we used linear regression. First, we corrected for possible spatial gradients within the study area (such as related to

climate). We used the x and y coordinates of the centers of abandoned fields and their interaction (x*y) as independent variables (Table 2) and tested their effects on the number of target species in the abandoned fields. The significant coordinates were thus used as covariates in selecting habitat characteristics. Some habitat characteristics were strongly correlated with each other (Appendix 2 in Electronic supplementary material). Therefore, we selected only the most important ones by bi-directional step-wise selection based on the AIC (Akaike Information Criterion, a form of penalized log-likelihood analysis, Crawley 2002).

Finally, we tested the overall effect of isolation of the abandoned fields, I_A (i.e., calculated based on the area of surrounding grasslands) or I_S (i.e., calculated based on the species richness of surrounding grasslands), on the number of target species occurring in the abandoned fields. To test the pure effect of isolation, pre-selected coordinates and habitat characteristics were used as covariates.

All of the analyses were performed in S-Plus 2000 (MathSoft 1999).

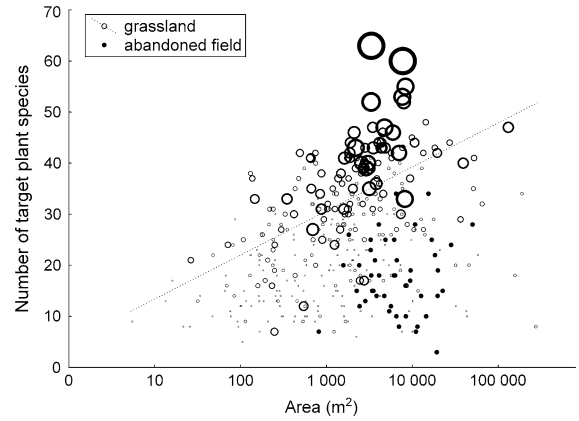


Fig. 1 Relationship between habitat area and the number of target species in grasslands and in abandoned fields. The size of the *open circles* denotes the number of grassland specialists (species not found in abandoned fields). Grasslands with no specialists are depicted as *small dots*. A *regression line* is shown for the full range of grasslands ($F_{1,337} = 68.85$, $R^2 = 0.170$, $P < 0.001$); the species–area relationship was not significant for abandoned fields ($F_{1,44} = 0.047$, $P = 0.829$) or for grasslands when using the same span of areas as for abandoned fields ($F_{1,206} = 3.12$, $P = 0.078$)

Results

We found 59 out of the 87 (i.e., 68%) target dry grassland species in the abandoned fields. Twenty-eight species were not found in the abandoned fields and were therefore considered to be grassland specialists (Table 1).

The number of target species in each abandoned field varied from 3 to 34 (mean 18), whereas the number of target species in the dry grasslands varied from 5 to 63 (mean 26). When considering only generalists (species occurring in at least one abandoned field), the number of target species occurring in the dry grasslands varied from 3 to 47 (mean 24). Grasslands harbored significantly more target species than abandoned fields when considering both generalists and specialists ($F_{1,382} = 55.88$, $P < 0.001$, $R^2 = 0.12$) or generalists alone ($F_{1,382} = 47.66$, $P < 0.001$, $R^2 = 0.10$; Fig. 1).

A significantly positive relationship between the number of target species in dry grasslands and the logarithm of grassland area was found only when analyzing the whole range of grasslands (Fig. 1; $F_{1,337} = 68.85$, $R^2 = 0.170$, $P < 0.001$ for all target species; $F_{1,337} = 70.320$, $R^2 = 0.173$, $P < 0.001$ for generalists; $F_{1,337} = 22.840$, $R^2 = 0.063$, $P < 0.001$ for specialists) However, the species–area relationships were relatively weak, indicating differences in habitat quality between individual grasslands. The weakest species–area relationship was found when it was calculated only for specialists. However, when we considered only grasslands within the same span of areas as the abandoned fields, the species–area relationship was not significant ($F_{1,206} = 3.12$, $P = 0.078$ for all species; $F_{1,206} = 3.53$, $P = 0.06$ for generalists; $F_{1,206} = 0.66$, $P = 0.419$ for specialists), nor was the relationship between the number of target species occurring in abandoned fields and field area ($F_{1,44} = 0.047$, $P = 0.829$).

All of the three tested spatial coordinates (x, y and x*y) and seven out of 13 habitat characteristics were selected in a stepwise analysis in the most parsimonious model explaining the number of target species in abandoned fields (Table 2). We found a negative effect of seeding and PDSI in March and June and positive effects of area, slope and PDSI in May (Table 2). The topographic wetness index (TWI) and field age were not included in final model.

More target species always occurred in less isolated abandoned fields. When habitat characteristics were used as covariates, the number of target species in

Table 3 Variability explained by the two measures of isolation in different models

Habitat characteristics	Distance	Source habitats	I_A			I_S		
			R^2	F	P	R^2	F	P
Included	Edge-to-edge	Grasslands	**0.05**	**4.38**	**0.046**	**0.07**	**6.19**	**0.019**
		Grasslands + abandoned fields	–	0.47	0.500	–	1.24	0.275
	Center-to-center	Grasslands	–	0.56	0.460	–	2.55	0.122
		Grasslands + abandoned fields	–	0.19	0.663	–	0.12	0.732
Not included	Edge-to-edge	Grasslands	–	1.76	0.191	**0.10**	**4.82**	**0.033**
		Grasslands + abandoned fields	–	1.18	0.284	**0.10**	**4.95**	**0.031**
	Center-to-center	Grasslands	–	1.90	0.175	**0.16**	**8.54**	**0.005**
		Grasslands + abandoned fields	–	1.14	0.291	**0.12**	**6.16**	**0.017**

I_A was based on the area of source habitats, and I_S was based on the number of target species in the source habitats. Habitat characteristics were selected in step-wise regression (Table 2)

Values in bold are significant on $P < 0.05$

abandoned fields was significantly affected by I_A (calculated using the area of source habitats) and I_S (calculated using the species richness of target species on source habitats) only when edge-to-edge distance and grasslands alone as source habitats were used for the calculations (Table 3). Isolation I_S explained 34% more variability in the number of target species in abandoned fields than isolation I_A. In contrast, when habitat characteristics were not included, isolation I_S always had a significant effect on the number of target species in abandoned fields, and the effect of I_A was never significant (Table 3). The effect of isolation was generally more pronounced when only grasslands were used as source habitats. The explained variability also differed between models using different measures of distance (edge-to-edge and center-to-center; Table 3).

Discussion

Habitat isolation is known to be an important factor reducing the species richness of dispersal-limited taxa in a wide range of habitats. For grassland plants, however, the effect of present habitat isolation on species richness is often thought to be obscured by land use history (Lindborg and Eriksson 2004; Helm et al. 2006; Gustavsson et al. 2007). Even highly isolated grasslands might be species rich due to the longevity and persistence of species from a time when the grassland was larger and/or more connected to other grasslands. Here, we studied the colonization patterns of grassland species in recently abandoned fields, and the distribution of dry grassland species in these habitats should only reflect the present landscape structure. We showed that the effect of isolation strongly depends on the inclusion of habitat characteristics in the model as well as on the parameters used when calculating isolation.

The new approach developed in the present study for measuring isolation based on the species richness of source habitats (I_S) always produced better results than a commonly used area-based measure (I_A). This confirmed our hypothesis that the area of surrounding grasslands may not fully reflect their importance as a source of propagules. The use of area-based isolation (I_A) is justified by the fact that larger habitats are generally richer in species and host larger populations. However, in many previous studies in grasslands (e.g., Eriksson and Ehrlen 2001; Lindborg and Eriksson 2004; Bruun 2005; Helm et al. 2006; Lobel et al. 2006), species-area or population size–area relationships were found to be weak or even non-existent. Similarly, our results revealed that the most species-rich grasslands were not the largest by far (Fig. 1), suggesting that factors other than area are more important for the species richness of the grasslands in our study system.

To deal with differences among propagule sources, Ruprecht (2006) weighted the areas of source grasslands according to their phytosociological classification. However, this type of approach relies on division of habitats into several categories and may not be optimal. We suggest that species richness might be a better proxy of habitat importance in terms of propagule sources than vegetation type, and our new

measure of isolation based on the species richness of source habitats (I_S) can be more widely applied.

It is likely that isolation could be interconnected with important habitat characteristics. Where conditions are more suitable for grassland species, grasslands might be more abundant and clumped and therefore less isolated compare to areas with less suitable conditions. Indeed, when habitat characteristics were included, Lobel et al. (2006) did not demonstrate an effect of the isolation of grassland fragments on plant species richness, and Adriaens et al. (2006) found only a marginal effect of habitat isolation on a few functional of groups of plant species. Other studies demonstrating the effect of habitat isolation on the species richness in grasslands have not taken habitat characteristics into account (e.g., Reitalu et al. 2009; Bruckmann et al. 2010). Our results confirm that inclusion of habitat characteristics as covariates might considerably alter conclusions regarding the effect of isolation.

The relative importance of isolation also changed when different measurements of distance were applied. When calculating the isolation of individual abandoned fields, we considered all source habitats within a distance of less than 0.5 km. As a consequence, when using edge-to-edge distance, we included 3–4 more source habitats on average than when using center-to-center distance. Therefore, isolation calculated using edge-to-edge distance was systematically lower than isolation based on center-to-center distance. The greater amount of variability explained by isolation based on center-to-center than on edge-to-edge distance suggests that grassland plants are limited by dispersal at distances even shorter than 0.5 km. Nevertheless, the above-mentioned pattern only held when habitat characteristics were not included in the model. In contrast, isolation based on edge-to-edge distance was the only significant isolation measure when covariates were included. Edge-to-edge isolation measures isolation at a larger spatial scale, and it may therefore not be as strongly affected by the habitat conditions of the sites as is the center-to-center measure of isolation.

The populations of target species found in abandoned fields typically consisted of a few individuals to tens of individuals, and their long-term prospects are therefore uncertain. Nevertheless, even transient populations might positively influence species persistence at the landscape scale (Loehle 2007). Our results thus confirm the importance of abandoned fields in the landscape dynamics of dry grassland species. Two-thirds of the target species were able to become established and could potentially spread farther in the landscape. However, when we used abandoned fields as additional source habitats for calculating isolation, the resulting effect of isolation was almost always less pronounced than when using only grasslands as source habitats. The importance of abandoned fields as sources of propagules of dry grassland species is therefore rather low in the study area.

There is increasing evidence that different processes control the species richness as well as the distributions of specialist and generalist species (Pandit et al. 2009) and that these two groups of species respond differently to landscape changes (With and Crist 1995). Bartha et al. (2003) showed that the influx of new colonizers was highest in the first 5–6 years after field abandonment, with much lower numbers of new species appearing later. The fields surveyed in our study were abandoned a maximum of 15 years ago and we can therefore expect that most of the species with the ability to colonize the abandoned fields would have already done so. Therefore, our definition of grassland specialists (species that were not able to colonize any of the abandoned fields in the time span of 15 years) to some degree reflects poor dispersal abilities of species together with narrow habitat requirements.

The difference in the strength of the species–area relationship between specialists and generalists confirms that there is an obvious difference between the landscape dynamics of the two groups and that our division is not arbitrary. Moreover, relaxed species–area relationship of specialists in grasslands implies that habitat quality is more important for their distribution than habitat area. Habitat quality might be associated with certain specific habitat conditions, either abiotic (such as pH or water holding capacity; Munzbergová 2004; Lobel et al. 2006) or biotic (such as character of soil biota, Hartnett and Wilson 1999; van der Heijden et al. 2008). Both biotic and abiotic conditions are likely to be influenced by the land-use history of a site (e.g., Karlik and Poschlod 2009; Oehl et al. 2010; Postma-Blaauw et al. 2010). We therefore suggest that similar to "ancient forest species" (Honnay et al. 1998; Hermy et al. 1999), our specialists are restricted to grasslands with long continuity of land use. However, this needs to be further tested. Our results nevertheless show that pure presence-absence

surveys carried out in grasslands and fields may help to distinguish the most specialized species, which are likely to suffer most from the loss of their habitat.

In our extensive field survey, two-thirds of the target dry grassland species were found in fields abandoned in the last two decades. However, the number of target species in abandoned fields was still significantly lower than the number of target species found in grasslands. Moreover, the difference was not due to specialists missing in the abandoned fields, it holds also when compared only numbers of generalist species on fields and grasslands. This implies that even for those species that can reach abandoned fields, some limitations still exist.

The suitability of abandoned fields for dry grassland species may be decreased by numerous factors. Because the simple species–area relationship was not significant for the abandoned fields, we assume that grassland plants do not benefit from the greater heterogeneity of larger fields because they are only able to colonize a few, more open sites. The extent of these suitable sites within each field is influenced more by other habitat characteristics than by field area. We assume the strongest negative effect to be associated with vigorously growing grass and weedy species. Indeed, fewer target species were found in fields that were seeded with a commercial seed mixture prior abandonment resulting in the formation of dense vegetation cover. Similarly, in steeper fields, nutrients are washed away faster than in flat fields, and vegetation becomes less dense and more hospitable for new colonizers.

Conclusion

We revealed an unexpectedly high success of grassland species in the colonization of fields abandoned in the last two decades. These abandoned fields may thus play an important role in the landscape dynamics of many grassland species, but their dispersal is probably often limited to short distances (less than 0.5 km). The proximity of species-rich rather than large source habitats was shown to be important for field colonization. Our new isolation measure using the distance and species richness of surrounding habitats may be helpful in studying the effects of landscape structure on species richness in landscapes with pronounced effects of land-use history or other important factors reducing the species–area relationship.

Assessing the species richness of source habitats would appear to be much more time-consuming than just calculating their areas. However, in the majority of studies addressing habitat isolation, there is no distinction made between target and source habitats, and species richness is known for all habitats under study to be the main response variable. Therefore, there is no additional effort needed for this type of assessment (except for substitution into a formula), and our approach might be easily applied and further tested.

We have also shown that inclusion of habitat characteristics as covariates may considerably alter conclusions about the effects of isolation, which could actually be overestimated when assessed separately. We therefore highly recommend considering habitat characteristics when studying habitat isolation. Through comparison of results obtained with and without inclusion of habitat characteristics, we may gain novel insights into factors affecting species richness.

Acknowledgments We thank Ondra Beran, Zita Cervenkova, Michal Knapp, Dana Pruchova, Katka Tremlova and Jana Zmeskalova for their help in the field. We are grateful to Michal Knapp, people from the Population Ecology discussion group in Pruhonice and two anonymous referees for valuable comments on previous versions of the manuscript. This study was supported by the Grant Agency of Czech Republic (Grant no. P505/10/0593 and partly also no. 206/08/H049), and partly by the Ministry of Education, Youth and Sports of the Czech Republic (Grant no. 0021620828) and Academy of Sciences of the Czech Republic (Grant no. AV0Z60050516).

References

Adriaens D, Honnay O, Hermy M (2006) No evidence of a plant extinction debt in highly fragmented calcareous grasslands in Belgium. Biol Conserv 133(2):212–224

Bartha S, Meiners SJ, Pickett STA, Cadenasso ML (2003) Plant colonization windows in a mesic old field succession. Appl Veg Sci 6(2):205–212

Bennie J, Hill MO, Baxter R, Huntley B (2006) Influence of slope and aspect on long-term vegetation change in British chalk grasslands. J Ecol 94(2):355–368

Bruckmann SV, Krauss J, Steffan-Dewenter I (2010) Butterfly and plant specialists suffer from reduced connectivity in fragmented landscapes. J Appl Ecol 47(4):799–809

Bruun HH (2005) A field test of the relationship between habitat area and population size for five perennial plant species. Web Ecol 5:1–5

Chylova T, Munzbergova Z (2008) Past land use co-determines the present distribution of dry grassland plant species. Preslia 80(2):183–198

Cook WM, Yao J, Foster BL, Holt RD, Patrick LB (2005) Secondary succession in an experimentally fragmented landscape: community patterns across space and time. Ecology 86(5):1267–1279

Cousins SAO (2001) Analysis of land-cover transitions based on 17th and 18th century cadastral maps and aerial photographs. Landscape Ecol 16(1):41–54

Cousins SAO (2009a) Landscape history and soil properties affect grassland decline and plant species richness in rural landscapes. Biol Conserv 142(11):2752–2758

Cousins SAO (2009b) Extinction debt in fragmented grasslands: paid or not? J Veg Sci 20(1):3–7

Cousins SAO, Aggemyr E (2008) The influence of field shape, area and surrounding landscape an plant species richness in grazed ex-fields. Biol Conserv 141(1):126–135

Cousins SAO, Eriksson O (2008) After the hotspots are gone: land use history and grassland plant species diversity in a strongly transformed agricultural landscape. Appl Veg Sci 11(3):365–374

Crawley MJ (2002) Statistical computing: An introduction to data analysis using S-Plus. Wiley, Chichester

Ellenberg H (1988) Vegetation ecology of Central Europe. Cambridge University Press, Cambridge

Eriksson O, Ehrlen J (2001) Landscape fragmentation and the viability of plant populations. In: Silvertown J, Antonovics J (eds) Integrating ecology and evolution in spatial context. Blackwell, Oxford, pp 157–175

Eriksson A, Eriksson O, Berglund H (1995) Species abundance patterns of plants in Swedish seminatural pastures. Ecography 18(3):310–317

Eriksson O, Cousins SAO, Bruun HH (2002) Land-use history and fragmentation of traditionally managed grasslands in Scandinavia. J Veg Sci 13(5):743–748

ESRI (2006) ArcGIS 9.2. Environmental Systems Research Institute, Redlands

Gustavsson E, Lennartsson T, Emanuelsson M (2007) Land use more than 200 years ago explains current grassland plant diversity in a Swedish agricultural landscape. Biol Conserv 138(1–2):47–59

Hanski I (1994) A practical model of metapopulation dynamics. J Anim Ecol 63(1):151–162

Hanski I (1999) Habitat connectivity, habitat continuity, and metapopulations in dynamic landscapes. Oikos 87(2):209–219

Hartnett DC, Wilson GWT (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. Ecology 80(4):1187–1195

Helm A, Hanski I, Partel M (2006) Slow response of plant species richness to habitat loss and fragmentation. Ecol Lett 9(1):72–77

Hermy M, Honnay O, Firbank L, Grashof-Bokdam C, Lawesson JE (1999) An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. Biol Conserv 91(1):9–22

Honnay O, Degroote B, Hermy M (1998) Ancient-forest plant species in Western Belgium: a species list and possible ecological mechanisms. Belg J Bot 130(2):139–154

Janssens F, Peeters A, Tallowin JRB, Bakker JP, Bekker RM, Fillat F, Oomes MJM (1998) Relationship between soil chemical factors and grassland diversity. Plant Soil 202(1):69–78

Karlik P, Poschlod P (2009) History or abiotic filter: which is more important in determining the species composition of calcareous grasslands? Preslia 81(4):321–340

Kindlmann P, Burel F (2008) Connectivity measures: a review. Landscape Ecol 23(8):879–890

Kiviniemi K (2008) Effects of fragment size and isolation on the occurrence of four short-lived plants in semi-natural grasslands. Acta Oecol 33(1):56–65

Kiviniemi K, Eriksson O (1999) Dispersal, recruitment and site occupancy of grassland plants in fragmented habitats. Oikos 86(2):241–253

Lindborg R, Eriksson O (2004) Historical landscape connectivity affects present plant species diversity. Ecology 85(7):1840–1845

Lindborg R, Cousins SAO, Eriksson O (2005) Plant species response to land use change—*Campanula rotundifolia*, *Primula veris* and *Rhinanthus minor*. Ecography 28(1):29–36

Lipsky Z (1995) The changing face of the Czech Rural landscape. Landsc Urban Plan 31(1–3):39–45

Lobel S, Dengler J, Hobohm C (2006) Species richness of vascular plants, bryophytes and lichens in dry grasslands: the effects of environment, landscape structure and competition. Folia Geobot 41(4):377–393

Loehle C (2007) Effect of ephemeral stepping stones on metapopulations on fragmented landscapes. Ecol Complex 4:42–47

Luoto M, Rekolainen S, Aakkula J, Pykala J (2003) Loss of plant species richness and habitat connectivity in grasslands associated with agricultural change in Finland. Ambio 32(7):447–452

MathSoft Inc (1999) S-Plus 2000 Professional Release 2.Data Analysis Products Division. MathSoft, Seattle

Moilanen A, Nieminen M (2002) Simple connectivity measures in spatial ecology. Ecology 83(4):1131–1145

Munzbergova Z (2004) Effect of spatial scale on factors limiting species distributions in dry grassland fragments. J Ecol 92(5):854–867

Oehl F, Laczko E, Bogenrieder A, Stahr K, Bosch R, van der Heijden M, Sieverding E (2010) Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biol Biochem 42(5):724–738

Oster M, Cousins SAO, Eriksson O (2007) Size and heterogeneity rather than landscape context determine plant species richness in semi-natural grasslands. J Veg Sci 18(6):859–868

Oster M, Ask K, Cousins SAO, Eriksson O (2009a) Dispersal and establishment limitation reduces the potential for successful restoration of semi-natural grassland communities on former arable fields. J Appl Ecol 46(6):1266–1274

Oster M, Ask K, Romermann C, Tackenberg O, Eriksson O (2009b) Plant colonization of ex-arable fields from adjacent species-rich grasslands: the importance of dispersal vs recruitment ability. Agr Ecosyst Environ 130(3–4):93–99

Pandit SN, Kolasa J, Cottenie K (2009) Contrasts between habitat generalists and specialists: an empirical extension to the basic metacommunity framework. Ecology 90(8): 2253–2262

Partel M, Zobel M (1999) Small-scale plant species richness in calcareous grasslands determined by the species pool, community age and shoot density. Ecography 22(2): 153–159

Poschlod P, Bonn S (1998) Changing dispersal processes in the central European landscape since the last ice age: an explanation for the actual decrease of plant species richness in different habitats? Acta Bot Neerl 47(1):27–44

Postma-Blaauw MB, de Goede RGM, Bloem J, Faber JH, Brussaard L (2010) Soil biota community structure and abundance under agricultural intensification and extensification. Ecology 91(2):460–473

Prugh LR (2009) An evaluation of patch connectivity measures. Ecol Appl 19(5):1300–1310

Pykala J, Luoto M, Heikkinen RK, Kontula T (2005) Plant species richness and persistence of rare plants in abandoned semi-natural grasslands in northern Europe. Basic Appl Ecol 6(1):25–33

Pywell RF, Bullock JM, Hopkins A, Walker KJ, Sparks TH, Burke MJW, Peel S (2002) Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. J Appl Ecol 39(2):294–309

Reitalu T, Sykes MT, Johansson LJ, Lonn M, Hall K, Vandewalle M, Prentice HC (2009) Small-scale plant species richness and evenness in semi-natural grasslands respond differently to habitat fragmentation. Biol Conserv 142(4):899–908

Ruprecht E (2006) Successfully recovered grassland: a promising example from Romanian old-fields. Restor Ecol 14(3):473–480

Tremlova K, Munzbergova Z (2007) Importance of species traits for species distribution in fragmented landscapes. Ecology 88(4):965–977

Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds) (1964–1983). Flora Europaea. Cambridge University Press, New York

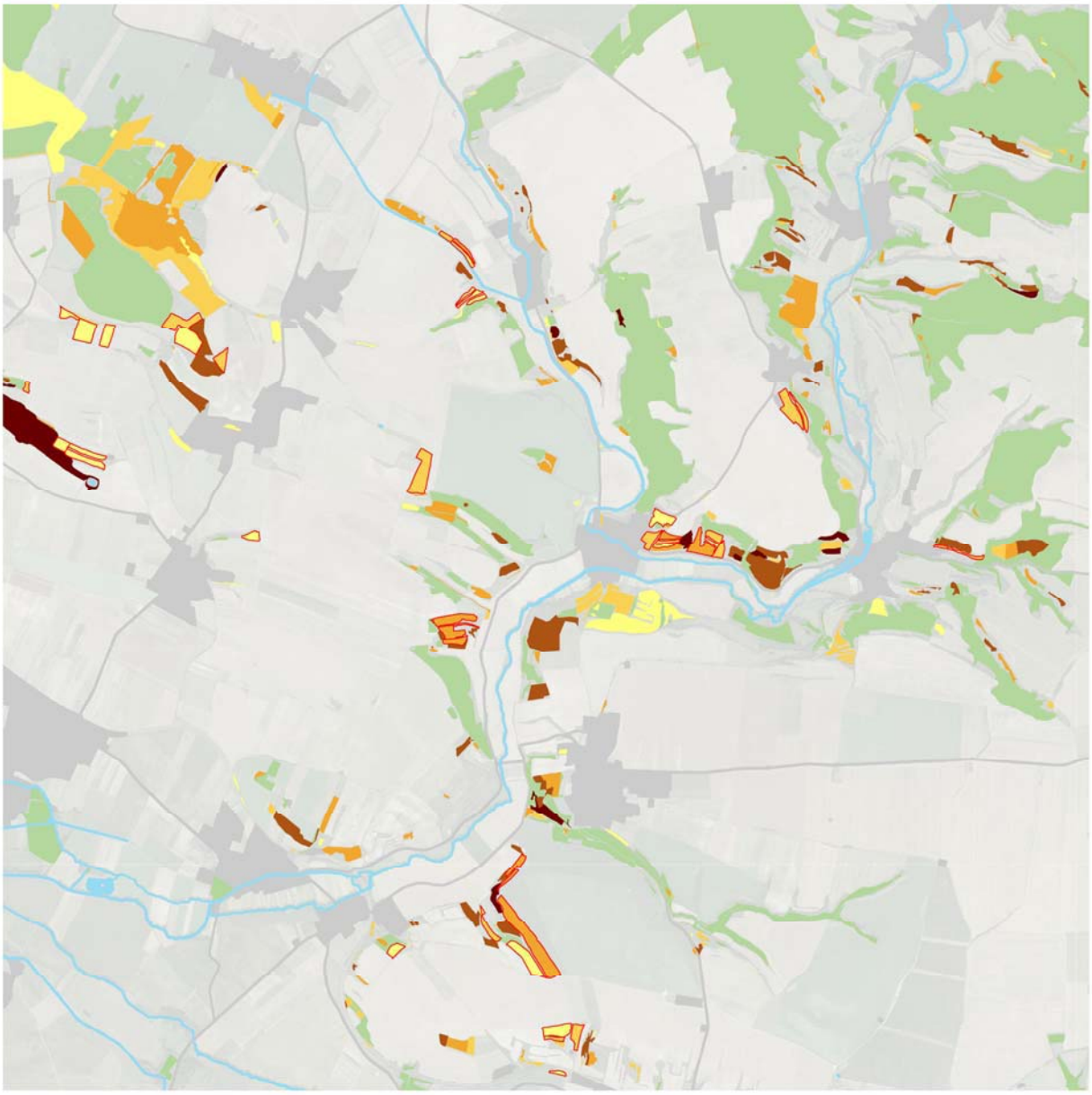
van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11(3):296–310

Walker KJ, Stevens PA, Stevens DP, Mountford JO, Manchester SJ, Pywell RF (2004) The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. Biol Conserv 119(1):1–18

Web 1. http://portal.chmi.cz/. Downloaded 26 Apr 2011

With KA, Crist TO (1995) Critical thresholds in species responses to landscape structure. Ecology 76(8):2446–2459

Appendix 1 - Map of the study area



Appendix 2 – Detailed information on the calculation of habitat characteristics

For the whole study area, we constructed a digital elevation model (DEM) with a 10 m grid size based on digital contour lines (1:10 000, 2 m vertical distance) provided by the Czech Office for Surveying, Mapping and Cadastre. As a gridding technique, we used ANUDEM (Hutchinson 1989) implemented in ArcGIS 9.2 (ESRI 2006). Based on the DEM, we created grids of slopes and potential direct solar irradiation (PDSI) for the 21st day of December to June using ArcGIS 9.2 (ESRI 2006).

We also calculated the topographic wetness index (TWI) using SAGA (Web 1) based on the slope grid and specific catchment area grid as TWI = ln (*As*/tan *b*), where *As* is the specific catchment area (the cumulative upslope area draining through a cell divided by the contour width), and *b* is the local slope (Beven and Kirkby 1979). As suggested by the critical study performed by Kopecky and Cizkova (2010), we used the method of Zevenbergen and Thorne (1987) to create the slope grid and the multiple flow routing algorithms of Quinn et al. (1991) to create the specific catchment area grid.

For each abandoned field, we calculated mean values of the slope, TWI and PDSI for December through June. The slope varied between 3-17° with a median of 8.5°. TWI is a non-dimensional index used to describe spatial soil moisture patterns. When simplified, higher TWI values denote higher soil moisture. In our study, TWI reached values between 7.7-14.5 with a median of 9.4. PDSI sums up the radiation per square meter at a particular site throughout day. In the study area it is lowest in December, when it varies between 121 and 274 $kw.m^{-2}.day^{-1}$ (median 417 $kw.m^{-2}.day^{-1}$), and reaches maximum in June, when it varies between 5205 and 5791 $kw.m^{-2}.day^{-1}$ (median 5702 $kw.m^{-2}.day^{-1}$).

We also used digital geological data for bedrock (GEOCR 25, 1:25 000 provided by the Czech Geological Survey) to assess the relative extent of each bedrock type in each abandoned field. Eight bedrock type categories occurred in the area: sandstone, sediment, loess + loess loam, sand, loam, gravel, claystone + marlite + siltstone and limestone. For each field, the percentage of each bedrock type was assessed, summing to 1.

References:

Beven, K.J. and Kirkby, M.J. (1979) A physically based, variable contributing area model of basin hydrology. Hydrolog Sci Bull 24, 43–69.

ESRI (2006) ArcGIS 9.2. Environmental Systems Research Institute. Redlands.

Hutchinson, M.F. (1989) A new procedure for gridding elevation and stream line data with automatic removal of spurious pits. J Hydrol 106(3-4), 211–232.

Kopecky, M. and Cizkova, S. (2010) Using topographic wetness index in vegetation ecology: does the algorithm matter? Appl Veg Sci, 13(4), 450-59.

Quinn, P., Beven, K., Chevallier, P. and Planchon, O. (1991) The prediction of hillslope flow paths for distributed hydrological modeling using digital terrain models. Hydrol Process 5(1), 59–80.

Web1 http://www.saga-gis.org/en/index.html, 25. 1. 2011

Zevenbergen, L.W. Thorne, C.R. (1987) Quantitative-Analysis of Land Surface-Topography. Earth Surf Proc Land, 12(1), 47-56.

Appendix 3 - Pair wise correlations of all parameters of abandoned fields. R values are shown. Significant correlations ($P < 0.05$) are in bold.
x, y = spatial coordinates, PDSI = potential direct solar irradiation, TWI = topographic wetness index

	x	y	x*y	Age	Seeding	Area	TWI	Slope	PDSI December	PDSI January	PDSI February	PDSI March	PDSI April	PDSI May	PDSI May	Sandstone	Sediment	Loess + loess loam	Clay	Sand	Gravel	Clay stone + marlite + siltstone	Limestone
y	-0.28																						
x*y	**0.71**	**0.46**																					
Age	**0.46**	-0.04	**0.41**																				
Seeding	**0.37**	0.16	**0.47**	**0.51**																			
Area	-0.2	0.01	-0.18	-0.09	0.07																		
TWI	-0.23	-0.22	**-0.36**	0.02	-0.18	-0.12																	
Slope	**0.38**	**0.31**	**0.55**	0.09	0.23	-0.28	**-0.67**																
PDSI December	0.04	**0.36**	0.25	-0.06	0.24	-0.08	**-0.5**	**0.44**															
PDSI January	0.05	**0.38**	0.26	-0.07	0.23	-0.09	**-0.48**	**0.43**	**1**														
PDSI February	0.05	**0.38**	0.26	-0.07	0.23	-0.09	**-0.47**	**0.42**	**1**	**1**													
PDSI March	0.03	**0.38**	0.24	-0.08	0.23	-0.08	**-0.45**	**0.39**	**1**	**1**	**1**												
PDSI April	-0.04	**0.38**	0.18	-0.1	0.22	-0.03	**-0.41**	**0.3**	**0.98**	**0.99**	**0.99**	**0.99**											
PDSI May	-0.09	**0.37**	0.13	-0.11	0.21	0.01	**-0.38**	0.22	**0.97**	**0.97**	**0.97**	**0.98**	**1**										
PDSI June	-0.15	**0.37**	0.07	-0.13	0.2	0.06	**-0.34**	0.14	**0.94**	**0.94**	**0.95**	**0.96**	**0.98**	**1**									
Sandstone	**0.63**	**-0.43**	0.29	0.16	**0.35**	0.07	-0.2	0.02	0.06	0.05	0.05	0.04	0.03	0.02	0.01								
Sediment	0.13	0.06	0.15	-0.14	-0.21	-0.16	0.27	-0.08	-0.12	-0.07	-0.06	-0.06	-0.07	-0.08	-0.08	-0.15							
Loess+ loess loam	0.06	0.24	0.24	-0.07	-0.03	-0.01	-0.09	-0.02	0.04	0.04	0.05	0.05	0.05	0.05	0.05	-0.22	0.07						
Clay	**-0.35**	0.14	-0.21	0.06	-0.2	-0.01	-0.04	0.15	-0.06	-0.07	-0.08	-0.08	-0.09	-0.09	-0.09	**-0.64**	-0.21	-0.17					
Sand	-0.15	-0.04	-0.14	**0.3**	-0.09	-0.2	0.09	0.13	-0.19	-0.2	-0.2	-0.21	-0.24	-0.26	-0.28	**-0.51**	-0.18	-0.14	**0.74**				
Gravel	**-0.35**	0.14	-0.21	0.06	-0.2	-0.01	-0.04	0.15	-0.06	-0.07	-0.08	-0.08	-0.09	-0.09	-0.09	**-0.64**	-0.21	-0.17	**1**	**0.74**			
Clay stone + marlite + siltstone	**-0.44**	0.26	-0.27	-0.14	-0.18	0.12	0.26	-0.23	0.09	0.08	0.09	0.1	0.13	0.16	0.19	-0.28	-0.09	-0.09	-0.15	-0.16	-0.15		
Limestone	**-0.51**	0.22	**-0.32**	**-0.3**	-0.02	-0.02	-0.01	0.02	0.05	0.05	0.05	0.05	0.07	0.09	0.1	**-0.33**	-0.11	-0.1	0.04	-0.09	0.04	-0.02	
Isolation IA	-0.2	0	-0.21	-0.14	0.01	0.26	**-0.31**	0.24	0.2	0.19	0.18	0.18	0.18	0.18	0.18	-0.18	-0.21	-0.11	**0.35**	0.12	**0.35**	0.06	0.05
Isolation IS	-0.06	-0.13	-0.17	-0.09	-0.02	0.26	-0.27	0.17	0.1	0.09	0.09	0.09	0.08	0.08	0.08	-0.03	-0.21	-0.1	0.23	0.07	0.23	0.08	-0.12

Contrasting effects of resident vegetation on establishment, growth and reproduction of dry grassland plants on abandoned fields

Jana Knappová, Michal Knapp & Zuzana Münzbergová
manuscript

Contrasting effects of resident vegetation on establishment, growth and reproduction of dry grassland plants on abandoned fields

Jana Knappová[1,2], **Michal Knapp**[3], **Zuzana Münzbergová**[1,2]

[1]Department of Botany/Ecology, Faculty of Science, Charles University in Prague, Benátská 2, 128 01, Prague, Czech Republic
[2]Institute of Botany, Academy of Sciences of the Czech Republic, 252 43, Průhonice, Czech Republic
[3]Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, 165 21, Prague, Czech Republic

Abstract

Abandoned fields are perceived as alternative habitats for species from declining grasslands. Colonization of abandoned fields by grassland species might be constrained by both species dispersal and habitat conditions, which could be separated by seed sowing experiments.
Abandoned fields represent dynamic habitat with rapid vegetation changes. However, the effect of vegetation on sowing experiments assessing habitat limitation is only poorly known. Vegetation canopy might either compete with emerging seedlings or facilitate their establishment and it could differently affect seedlings and adult individuals. Moreover, the effects of vegetation might change with spatiotemporally variable factors such as weather or soil conditions. We aimed to test the effect of the resident vegetation of abandoned fields on the establishment, growth and reproduction of species typical of dry grasslands.
Seeds were sown in disturbed (bare of vegetation and roots) and undisturbed plots in three fields abandoned in the last 20 years. To assess the effects of temporal variation on plant establishment, we initiated our experiments in two years (2007 and 2008).
Almost one half of the 35 sown species reproduced within two years after sowing, while two species completely failed to become established. The vegetation in the undisturbed plots facilitated seedling establishment only in the year with low spring precipitation, and the effect did not hold for all species. In contrast, growth and reproduction were consistently much greater in the disturbed plots, but the effect size depended on soil properties of each field.
Abandoned fields provide suitable habitats for a number of grassland species, but the probability of establishing a viable and fertile population is largely constrained by the vigour of resident vegetation. Regarding the effect of resident vegetation on seedling establishment and growth, studies assessing habitat suitability should either involve both vegetation removal treatments and untreated, control plots or follow the gradient of vegetation cover. Seasonal variability involved in our study emphasizes the necessity of temporal replication of sowing experiments. We strongly recommend following the numbers of established individuals, their sizes and reproductive success to cover the entire plant life cycle when assessing habitat suitability.

Key-words: colonisation, competition, disturbance, facilitation, habitat suitability, plant traits, recruitment, seed addition, vegetation removal

Introduction

The extent of species-rich semi-natural grasslands has been drastically declining throughout Europe over the past century, and the remaining grasslands are fragmented and scattered within the landscape. Cessation of former management at some sites and management intensification at other sites has led to the deterioration of habitat conditions and decreased species richness (Eriksson et al., 2002; Luoto et al., 2003; Cousins, 2009). In recent decades, socio-economic changes and new technologies employed in agriculture have brought about the abandonment of arable fields that were no longer profitable to maintain (Cramer et al., 2008). Abandoned fields represent open, treeless habitats, and they are hence perceived as alternative habitats for species from declining grasslands (Walker et al., 2004). Indeed, many species are able to spontaneously colonise abandoned fields (Ruprecht, 2006; Öster et al., 2009a; Knappová et al., 2012). However, many other species are absent from communities that develop in abandoned fields and an important question remains why (Cramer et al., 2008; Fagan et al., 2008; Knappová et al., 2012).

The absence of particular species from a field can result either from the inability of species' propagules to reach the site (dispersal limitation) or from unsuitable conditions for a species to grow there (habitat limitation; Münzbergová & Herben, 2005). Whether current habitat conditions are limiting with respect to the successful establishment of a species can be determined by direct seed sowing (Turnbull et al., 2000; Münzbergová & Herben, 2005). Seed sowing experiments assessing habitat limitation are usually performed in relatively stable, mature communities, such as grasslands or forests (e.g., Münzbergová, 2004; Ehrlén et al., 2006; but see Öster et al., 2009a,b), and vegetation dynamics is not taken into account. Yet abandoned fields are highly dynamic systems with rapid changes in vegetation (Osbornová et al., 1990). High immigration rates can be usually observed in first years after field abandonment (Steffan-Dewenter & Tscharntke, 1997). Further colonization events of novel species are often connected with the collapse of resident vegetation (e.g., due to drought; Pakeman et al., 2002; Bartha et al., 2003). The above mentioned studies jointly demonstrate that resident vegetation has direct effects on establishment of novel species and therefore it could also considerably affect outcome of seed sowing experiments.

It has been repeatedly shown that resident plants compete with emerging seedlings (Burke & Grime, 1996; Tilman, 1997; Kosola & Gross, 1999; Kleijn, 2003). The resident vegetation, however, could also facilitate the establishment of other species (Connell & Slatyer, 1977; Brooker et al., 2008). The relative importance of competition and facilitation differs greatly among investigated species and environments (Gomez-Aparicio, 2009; Holmgren & Scheffer, 2010). Facilitating effects may turn into competitive interactions along with changes in important environmental factors, such as moisture or temperature (Veblen, 2008). For example, the effects of experimental disturbance on plant establishment can differ from one year to another depending on spring and summer precipitation (Bakker et al., 2003). Therefore, replication of sowing experiments in different years appears to be necessary to make any general conclusions about factors limiting species distribution, but this approach is surprisingly rare (Vaughn & Young, 2010; but see Špačková & Lepš, 2004; Seabloom, 2011).

Different developmental stages of the same species may exhibit different responses to competition from neighbours. For example, resident vegetation may

provide suitable microclimatic conditions for germination, but at the same time, it may reduce the growth and/or survival of germinated seedlings (Schupp, 1995; Howard & Goldberg, 2001; Fayolle et al., 2009). As a result the patterns observed in the early stages of plant development may not correspond to those observed in later developmental stages. Monitoring of seed sowing experiments should thus continue at least until the plants reach reproductive age (Turnbull et al., 2000). Therefore, we use numbers of established individuals, plant sizes and species reproduction success to express species' performance and response to resident vegetation.

The main aim of our study was (i) to determine the ability of a range of dry grassland species to become established after sowing on abandoned fields, (ii) to assess general effect of resident vegetation on species establishment, growth and reproduction and (iii) to determine role of seasonal variability on plant response to resident vegetation. To identify the extent to which the ability of species to colonise abandoned field is limited by species traits and habitat conditions, and whether this can be caused by resident vegetation at these sites we asked the following, more specific questions: (iv) Which plant traits can explain species-specific performance under different conditions? (v) Which site conditions modify the effect of vegetation on plant performance?

We hypothesize that species that are taller or have larger seeds are better adapted to withstand shading from vegetation and thus be less sensitive to surrounding vegetation. Reserves of larger seeds might also provide advantage in non-shaded disturbed plots in case of strong drought stress (Westoby et al., 1996). We also expect stronger suppressing effect of vegetation on sown species on sites which are richer in nutrients due to greater vigour of vegetation and stronger shading.

Methods

Study sites

The field seed sowing experiment was performed in the north-western part of the Czech Republic. The region is characterised by abundant fragments of species-rich calcareous dry grasslands (alliance *Bromion erecti*) surrounded mainly by arable fields. Some of these fields have been abandoned in the last two decades, and they are currently undergoing secondary succession. The long-term average temperature in the region is 7.7°C, and long-term normal precipitation is 612 mm (Web 1). Seed sowing took place in autumn 2007 and 2008, and most plants therefore germinated in spring 2008 and 2009, respectively. In 2008, May and June were abnormally dry, whereas in 2009, monthly precipitation levels from May to July were above the long-term normal (see Appendix S1 in Supporting Information).

Three fields abandoned in the last 20 years were chosen for the seed sowing experiment. All selected fields were overgrown with grasses and ruderal herbaceous vegetation. Unfortunately, we did not record detailed composition of resident vegetation prior sowing and subsequently two experimental fields were re-ploughed in the third year of experiment. Therefore, we could only report dominant species (see Appendix S2 in Supporting Information). It was not possible to assess the exact time since abandonment, since the ownership of the fields was unclear and no formal documentation exist about the former

management. However, we assume all the fields being approximately of the same age (i.e. abandoned 15-20 years ago).

To evaluate differences in habitat conditions between the three fields, data on soil properties were collected for each field. Six soil samples per block (see below for block definition) were taken in autumn 2007 and C (total, carbonate and organic), N, P, K, Ca and Mg concentrations and pH (in both water and KCl solutions) were analyzed in the laboratory (for methods, see e.g. Pánková *et al*., 2008). Additionally, we took 6 soil cores 100 cm2 per block to assess the maximum water holding capacity (Münzbergová, 2004).

Experimental set-up

At each experimental field, three blocks comprising of two disturbed and two undisturbed plots were established. One disturbed and one undisturbed plot in each block were sown at the end of November 2007, and the remaining two plots were sown at the end of November 2008. In the disturbed plots, the soil was trenched immediately prior to seed sowing to a depth of approximately 0.3 m, and turfs and roots were removed to minimise the resprouting of original vegetation from vegetative organs. In the undisturbed plots, no alterations were made prior to seed sowing. No further management (e.g., weeding of non-target species) was applied in the plots. Each sowing plot consisted of 36 squares of 0.33 x 0.33 m arranged in a rectangular grid of 1 x 4 m, which was surrounded by a 0.25 cm disturbed margin in the disturbed plots.

In summer and autumn prior to seed sowing, seeds of 35 species typical of dry grasslands in the region (Table 1) were collected from large populations in grasslands within 5 km from the experimental fields. All seeds were hand cleaned to maximise the number of ripe, viable seeds in the sample. Prior to sowing, seeds were stored at room temperature.

Each species was sown in a square that was randomly chosen within the sowing plot; only one species was sown in each square. With a few exceptions (Table 1), 100 seeds per species were sown per square. To assess the mean number of viable seeds sown per species, 3 × 100 seeds of each species were tested using a 0.1% solution of 2,3,5 - triphenyl tetrazolium chloride (Cottrell, 1947; Table 1).

Data collection

In September 2008-2010, all sowing plots were carefully examined, and all individuals of each species were counted in their respective squares. The number of flowering individuals was also assessed. To correct for natural regeneration, the mean number of individuals in squares adjacent to the sowing square of a particular species was recorded. The number of naturally recruited individuals was then subtracted from the number of individuals in the sowing square, and the resulting number was used instead. Although many dry grassland species occur in abandoned fields within the study area (Knappová *et al.*, 2012), natural regeneration in the sowing plots was negligible (zero in most species). Hereafter, we will use the terms first and second census to designate recordings with respect to the year of sowing. The first census of species sown in 2007 was performed in 2008, and the first census of species sown in 2008 was performed in 2009.

For a subset of species sown in 2008 (Table 1), the length of the longest leaf and the number of leaves were measured, since most species develop only earth-bound rosettes in a sterile form. Individual plant size was then assessed as leaf length × leaf number (as an approximation of plant biomass; McLellan, Law & Fitter, 1997). In most cases, all individuals of a respective species within each sowing square were measured. If a species was abundant, only 20 randomly chosen individuals were measured. Measurements were performed in two consecutive years (2009 and 2010). In the second census (in 2010), however, some plants had already started to flower, and no measurements comparable to those carried out for sterile plants were possible due to the different morphology of fertile and sterile plants. Therefore, the maximum size of sterile plants reached by particular species was assigned to each flowering plant for the purpose of our data analyses.

Data on species traits

We used data collected by Průchová (unpubl.) for the plant height and seed weight of all sown species. Plant height was assessed as the height of ten flowering plants randomly sampled in three populations within the study area (30 plants in total). Seed weight was measured for 50 seeds from three populations (150 seeds in total). Seed weight was log10 transformed prior to the analyses. We also used data on the Ellenberg indicator values expressing species requirements for nutrients, light, water, soil reaction, temperature and continentality of each species as species traits (Ellenberg *et al*., 1992).

Data analyses

We analysed data only for 18 species which were sown in both 2007 and 2008 (eight species were not) and were measured for plant size. We omitted species with too low germination rate (less than 1% of seeds germinating) or that were recorded in less than half of the plots (Table 1). This exclusion was necessary to avoid bias in results because the effects of studied factors were likely to be caused by chance in these excluded species.

At the first step, we used a generalised linear model (GLM) with a quasi-binomial distribution to analyse the effects of disturbance, species identity, locality and sowing year as well as their interactions on the number of established individuals. The dependent variable was number of individuals established within one sowing square expressed as the proportion of viable sown seeds of particular species (Table 1). Due to significant interaction of sowing year with all the three remaining variables (see Table S1 in Supporting Information), we subsequently performed the analyses separately for the two sowing years. Plant size and proportion of flowering individuals were analysed only for species sown in 2008. We also always analysed separately data from the first and second census.

To analyse the effects of disturbance, species identity, locality and their interactions on proportion of flowering individuals, we used a GLM with a quasi-binomial distribution. To analyse the effects of disturbance, species identity, locality and their interactions on plant size, we used a GLM with a Gaussian distribution. Plant size was $\log_{10}$ transformed prior to analyses. For the purpose of graphical presentation we used the relative plant size expressed as ratio between observed plant size (dependent variable in the analyses) and mean size of respective species across all plots and not the real plant size.

To analyse the effects of experimental treatments on plant size, we decided to use the maximum rather than the mean size per plot (i.e., the size of the largest plant of the respective species in the plot). We assumed that mean numbers would be strongly affected by mortality within the plots (small plants dying vs. small plants just surviving). In contrast, the maximum value refers to the potential size the species was able to achieve in a particular plot.

To assess the importance of species traits for species-specific response to disturbance, we performed the same set of analyses as described above (i.e., the analyses of data on the proportion of established individuals, the proportion of flowering individuals and plant size) on data based on sowing in 2008 with species identity being replaced by the value of particular trait of respective species. With this approach, we were able to assess what portion of variability explained by species is directly related to differences in particular traits among species.

Similarly, we wanted to assess the importance of habitat characteristics for spatial variability in the effect of disturbance. We first performed PCA of all soil parameters (each locality was a sample and data were standardized by "species"; Lepš & Šmilauer, 2003). Subsequently, locality was replaced by sample scores on first and second axes from this PCA in all the previous tests. With this approach, we were able to assess what portion of variability explained by locality is directly related to differences in soil condition at the localities.

We used a quasi-F criterion (ratio of the mean deviances of the explanatory variable and error term) for testing the significance of particular factors and their interactions (Francis *et al*., 1993). This enabled us to take the hierarchical structure of the data into account in the analyses. Species and all interactions with species were tested against residual variability (since each sown seed was replicate for species). Other factors were tested accordingly: disturbance against species × locality (× year), locality against disturbance × species (× year), and year against disturbance × species × locality. In this way, we took into account that disturbance was applied to each species at each locality in each year etc.

The experiment was arranged in blocks. Due to the structure of the data (only 3 blocks at only 3 localities), it was, however, not possible to include block as additional factor into the models (too low residual degrees of freedom). The blocks were thus used as a way to arrange the plots, but they were not considered in the tests. Nonetheless, when including block into the model without interactions, the relative importance of particular factors did not change (results not shown). All analyses were performed in R 2.11.0 (R Development Core Team, 2010) except for PCA, which was performed in Canoco for Windows 4.5 (Ter Braak & Šmilauer, 2002).

Results

Out of 35 sown species, 33 (nearly 95%) became successfully established in at least some localities or treatments (Table 1). The remaining two completely failed to become established. Seventeen out of the 35 species (almost 50%) started to flower in the second year following sowing, ten of which flowered exclusively in the disturbed plots (Table 1).

General effect of disturbance and temporal variability

Disturbance had significant negative effect on establishment for plants sown in 2007 and the effect was even stronger in the second census. In contrast, no significant effect of disturbance was detected for plants sown in 2008 (Table 2, Fig. 1a,b). Disturbance strongly influenced plant size in both censuses, with larger plants growing in the disturbed plots, although the effect was smaller in the second census (Table 2, Fig. 1c). Even greater positive effect of disturbance was detected in proportion of flowering individuals (Table 2, Fig. 1d). The year of sowing interacts with most other factors implying temporal variability in their effects (Table S1).

Inter-specific variability

Establishment success was strongly influenced by species identity, slightly more in plants sown in 2008 than in 2007 (Table 2). Even greater difference among species appeared in proportion of flowering individuals (Table 1 and Table

2). Contrary to the prevailing pattern, some species (e.g., *Agrimonia eupatoria, Salvia verticillata*) were more abundant in disturbed plots (Table 1), which probably contributed to the significant effect of the disturbance × species interaction on establishment. In contrast, all species consistently grew larger and flowered more in disturbed plots. Nevertheless, plant size was significantly affected by disturbance × species interaction in first census (Table 2).

None of the investigated plant traits significantly explained species-specific response to disturbance, neither in proportion of established individuals, nor in plant size ($P > 0.05$ in all cases).

Spatial variability

The effect of locality on establishment was only minor in plants sown in 2007 and considerable in plants sown in 2008 (Table 2, Fig. 2). Significant effect of locality in 2007 could be to large extent explained by differences in P, C, N, K and Mg concentrations correlated with first PCA axis of soil parameters (PCA1), whereas significant effect of locality in 2008 is mainly due to differences in WHC and pH correlated with second PCA axis (PCA2; Fig. 2, Appendix S2). Higher establishment in 2007 was associated with higher C, N, K, Mg and lower P concentrations; higher establishment in 2008 was associated with higher WHC and lower pH.

The effect of locality on plant size and on proportion of flowering individuals was relatively low (Table 2, Fig. 2). Spatial differences in plant size could be explained by both PCA axes, but the effect of PCA2 was stronger (Fig. 2). Greater plant size was associated with higher P concentrations and WHC, and with lower C, N, K, Mg concentrations and pH.

A weak significant effect of disturbance × locality interaction on establishment was found only in the second census in plants sown in 2007 (Table 2) and it could be partly explained by differences in WHC and pH (PCA2) among the fields. The effect of disturbance × locality interaction on plant size and flowering could be explained mostly by differences in N, P, C, Mg and K among the fields (PCA1), but the effect of PCA2 was also significant (Fig. 2). The significant interaction is given by the facts, that there was no difference in plant size among undisturbed plots on different fields whereas the effect of soil parameters on plant size was pronounced on disturbed plots.

The effect of species × locality interaction on establishment was quite high, albeit decreasing in time, for plants sown in 2007 and only slightly lower for plants sown in 2008 (Table 2). Similarly to disturbance × locality interaction, it could be partly explained by differences in WHC and pH among the fields (PCA2, Fig. 2). Similar pattern was found for the effect of species × locality interaction on plant size.

Discussion

The poor dispersal abilities of many grassland species and the disappearance of source populations are thought to hamper the colonisation of novel habitats, such as abandoned fields (Cramer et al., 2008; Öster et al., 2009a). When dispersal limitation is overcome (e.g., by means of seed sowing), species can be still limited by unsuitable conditions at a site (Münzbergová & Herben, 2005; Cramer et al., 2008). Our experiment demonstrated that many dry grassland species are in fact able to recruit, grow and even reproduce within two years,

when sown in abandoned fields suggesting that these fields should be considered to be suitable habitats for grassland species. However, we also found that conditions for both establishment and further growth are affected by resident vegetation indicating direct impact of vegetation on habitat suitability.

A number of studies have revealed higher seedling establishment rates in disturbed plots than under a vegetation canopy (e.g., Jakobsson & Eriksson, 2000; Zeiter et al., 2006; Hellström et al., 2009; Seabloom, 2011). The negative effect of resident vegetation is mainly attributed to the resulting increased competition for light (Burke & Grime, 1996; Jutila & Grace, 2002; Kleijn, 2003; Hofmann & Isselstein, 2004). In contrast, we found more seedlings in our undisturbed plots, suggesting a facilitative effect of vegetation on establishment. However, this positive effect was found only in species sown in 2007 and germinating in spring 2008, which was drier than normal. In contrast, above average precipitation was recorded in spring 2009 (especially in May; Appendix S1), when the establishment was comparable in disturbed and undisturbed plots (and even higher in disturbed plots in Field 1; Fig. 1). It is therefore likely that the effect of vegetation on seedling establishment is related to moisture. This is in agreement with the conclusion of Bakker et al. (2003), who documented a positive effect of May and July precipitation on seedling survival and differing effects of experimental management treatments depending on weather.

Positive effect of moisture on plant establishment in drier spring could also explain higher seedling numbers associated with higher WHC for plants sown in 2007. In the same study region, Münzbergová (2004) suggested that water availability and soil reaction limit seedling establishment and might be responsible for high β diversity within the studied dry grasslands. In contrast, higher seedling numbers of plants sown in 2008 were positively associated with N, C, Mg and K soil concentrations and negatively with P concentrations. This agrees with the findings of Janssens et al. (1998) who found phosphorus to have strong negative effect on plant recruitment and species diversity in grasslands. We are aware that the evidence on the effect of soil characteristics is weak because it is based on three experimental sites only. Moreover, the three experimental fields certainly differ in many other characteristics than those under study. Nonetheless, our results imply that habitat characteristics interact with other temporally variable conditions such as weather. In this light, temporal replication of sowing experiments is essential for drawing any general conclusions about factors limiting plant establishment.

Suitable conditions for establishment do not necessarily need to be suitable for growth, survival or reproduction (Schupp, 1995; Milbau et al., 2003). Indeed, resident vegetation significantly constrained the growth and flowering of established plants in our experiment although more individuals generally became established under a vegetation canopy than on bare ground. Moreover, the effect of disturbance on plant size was much stronger than on number of established individuals. Release from competition promoted plant growth and accelerated plant maturity, and larger plants were more likely to reproduce. Therefore, the opportunity for a species to successfully colonise an abandoned field (i.e., to establish and reproduce) depends to a large extent on the availability of open sites. Such open sites might originate e.g. from disturbances by animals (Olff & Ritchie, 1998; DeSimone & Zedler, 1999), from specific site conditions (e.g., on steeper slopes; Knappová et al., 2012) or from temporal collapse of whole vegetation canopy (e.g., due to drought; Pakeman et al., 2002; Bartha et al., 2003).

Importantly, successful establishment of grassland species is more likely also at the beginning of succession just after field abandonment, before canopy become closed (Steffan-Dewenter & Tscharntke, 1997).

The size of sown plants across the fields was uniformly low under vegetation canopy suggesting relatively strong competition from established plants. When released from competition in disturbed plots, plants vary in size depending on soil characteristics. In particular, larger plant size was associated with higher P concentrations and higher WHC. This implies that only in the absence of competitors, sown plants were able to benefit from higher phosphorus content and water supply. We can gain two important conclusions from these results. First, vegetation on studied abandoned fields rather homogenizes habitat conditions and makes fields generally inhospitable for grassland species. Second, the effect of phosphorus (and likely also the effect of other factors) is not consistent throughout the plant lifecycle.

Several plant traits have been proposed to be connected with enhanced performance under disturbance regimes or in competition of seedlings with established vegetation (Goldberg & Landa, 1991; Burke & Grime, 1996; Lavorel et al., 1999; Kahmen & Poschlod, 2004; Roberts et al., 2010; Lanta et al., 2011). Our failure to find any of plant traits to be related to species response to resident vegetation could be due to relatively small and homogenous group of investigated species.

It is also likely that several antagonistic mechanisms neutralize the effects of seed size. Larger seeds provide more reserves when species have to cope with unfavourable conditions, such as in shadow under vegetation canopy (Westoby, 1998). Large seeded species also present a longer germination time (Eriksson & Eriksson, 1997) and are hence less prone to fail to establish due to temporarily unfavourable conditions. However, at the same time, seed predators often favour larger seeds (Reader, 1993). Consequently, vegetation can indirectly negatively affect seedling establishment of large seeded species by providing habitat for seed predators (Bonser & Reader, 1998).

We also expected smaller plants to suffer more from competition of resident vegetation, but plant height did not explain species-specific reaction to disturbance, although larger plants generally performed better. This is likely due to relatively fast overgrowth of disturbed plots by non-sown resident species during the first year, which prevented smaller plants from benefiting from competition release.

Conclusions

We have demonstrated that abandoned fields provide suitable habitat for a number of grassland species and many even reached reproductive stage at these sites within the two years. We cannot assess whether the populations of sown species would be viable in the long term, but perennial plants might survive years or decades under unfavourable conditions (Helm et al., 2006; Gustavsson et al., 2007). Nevertheless, even if an abandoned field supports a population of a species for only a few years, such transient population might still positively influence species landscape dynamics and the persistence of the species at the landscape scale (Loehle, 2007).

However, the probability of establishing a viable population is constrained to large extent by the vigour of resident, often species poor and ruderal vegetation. Therefore, the effort should be directed to suppression of resident vegetation by

proper management (e.g., by mowing or grazing; Öster et al., 2009a) and/or to decreasing site productivity (e.g., by using hemiparasitic species; Pywell et al., 2004). The probability of establishing a viable population also considerably decreases with time following abandonment as succession proceeds and vegetation cover become more closed. Therefore, restoration efforts would be more effective if they take place immediately after field abandonment. Otherwise, more expensive interventions, such as tillage or harrowing, will be required to insure successful establishment of desirable species.

Seasonal variability involved in our study emphasizes the necessity of temporal replication of sowing experiments. Our results also highlight the importance of following the whole plant life cycle when assessing habitat suitability. Although this point has been stressed by some authors (Turnbull et al., 2000), it has been overlooked even in recent studies (e.g., Öster et al., 2009a). Regarding the effect of resident vegetation on seedling establishment and growth, studies assessing habitat suitability should either involve both vegetation removal treatments and untreated, control plots (e.g., Vítová & Lepš, 2011), or deliberately cover the widest range of canopy density within the studied habitat. Such an approach could provide novel insights into factors limiting species distribution.

Acknowledgements

We are grateful to L. Hemrová, A. Hradečná, J. Hradečný, V. Hrstková, K. Kadlecová, M. Michalík, H. Nutilová, J. Popelka, J. Švantnerová, K. Výborný, E. Warausová and D. Waraus for their assistance in the field, to K. Drtinová, V. Hradečná, A. Kovaříčková and R. Školník for preparing seeds before sowing and to people from PopEcol discussion group in Průhonice for their comments on manuscript draft. This work was financially supported by GAČR P505/10/0593, P504/10/0456 and partly by GAČR 206/08/H049, MŠMT 0021620828 and GAAV AV0Z60050516.

Table 1. List of all sown species. Sowing densities in absolute numbers and re-counted for fraction of viable seeds. Mean ± SD of established and flowering individuals is presented for second censuses. Each data point originates from three experimental fields with three sowing plots (replicates) per field. Species marked with asterisks were used in analyses. Nomenclature follows Tutin *et al*. (1964-80).

	Sown 2007		Established		Flowered		Sown 2008		Established		Flowered	
Species	Sown	Vital	disturbed	undisturbed	disturbed	undisturbed	Sown	Vital	disturbed	undisturbed	disturbed	undisturbed
*Agrimonia eupatoria**	100	88	9 ± 7.9	6.8 ± 5.3	0	0	100	74	13.1 ± 12.6	3.2 ± 3.5	1.4 ± 2	0
*Anthericum ramosum**	100	90	13 ± 6.6	23.2 ± 14.6	0	0	100	94	6.4 ± 7.5	4.4 ± 7.2	0	0
*Aster amellus**	100	32	0.3 ± 0.5	2.9 ± 2.8	0	0	100	60	0.7 ± 0.7	2.2 ± 3.2	0	0
Astragalus cicer	100	53	1.6 ± 0.8	3.9 ± 2.1	0.1 ± 0.3	0.2 ± 0.6	100	97	1 ± 1.5	1.2 ± 1.6	0	0
Astragalus glycyphyllos	-	-	-	-	-	-	100	98	4.4 ± 4.5	5.4 ± 4.7	0.1 ± 0.3	0
*Brachypodium pinnatum**	50	45	1.3 ± 1.2	5.1 ± 2.6	0	0	100	93	4.4 ± 2.9	3.3 ± 3.2	0.6 ± 1.1	0
*Bromus erectus**	25	21	2.7 ± 1.5	3.6 ± 1.9	0	0	100	42	8.2 ± 6.8	10.9 ± 7.9	1 ± 0.9	0
*Bupleurum falcatum**	100	51	2.9 ± 1.9	5.7 ± 3.9	1.6 ± 2.1	0	100	77	6.1 ± 2.8	7.1 ± 6.6	4.4 ± 2.7	0.2 ± 0.4
Carex flacca	100	17	0	0.6 ± 0.8	0	0	100	40	0	0	0	0
Carex tomentosa	100	20	0	0	0	0	100	22	0	0	0	0
*Carlina vulgaris**	100	85	4.4 ± 3.9	9.7 ± 7.6	0.2 ± 0.6	0	100	90	8.6 ± 5.1	7.3 ± 6.5	1.8 ± 2	0
*Centaurea jacea**	100	90	5 ± 3.1	10 ± 4.9	2.1 ± 2.2	0	100	88	9.3 ± 4.2	9.4 ± 5.3	5.2 ± 3	0.1 ± 0.3
*Centaurea scabiosa**	50	19	1.1 ± 1.4	2.3 ± 2.3	0.4 ± 0.8	0	100	80	6.4 ± 4.1	7.1 ± 6.7	0.3 ± 0.5	0
*Coronilla vaginalis**	70	63	1.7 ± 1.5	2.6 ± 1.3	0	0	50	44	1.1 ± 0.7	1 ± 1.4	0.1 ± 0.3	0
Coronilla varia	-	-	-	-	-	-	100	95	3.6 ± 2.5	3.4 ± 2.5	0.8 ± 0.8	0.9 ± 1.9
Filipendula vulgaris	100	39	0.4 ± 1	4.9 ± 4.6	0	0	100	0	0	0	0	0
Gentiana cruciata	-	-	-	-	-	-	100	88	0	0	0	0
Gobularia elongate	100	42	0.7 ± 1.1	0.6 ± 1	0	0	100	76	0.1 ± 0.3	0	0	0
Helianthemum nummularium subsp. *grandiflorum*	-	-	-	-	-	-	100	69	0	0	0	0
Inula salicina	100	24	0	0.1 ± 0.3	0	0	100	82	0	0.1 ± 0.3	0	0
*Linum tenuifolium**	100	45	2.7 ± 2.4	3.8 ± 4.2	0	0	100	25	0.7 ± 0.8	0.7 ± 1.2	0.3 ± 0.7	0.3 ± 0.7
Lotus corniculatus	100	59	2 ± 1.5	6.3 ± 3.4	0.4 ± 0.7	1.2 ± 1.9	100	94	4.1 ± 3.1	6.4 ± 3.7	3.1 ± 2.4	5.6 ± 4.2
Odontites lutea	100	35	0.6 ± 0.8	1.8 ± 3.6	0	0	-	-	-	-	-	-

Onobrychis viciifolia	100	56	4.7 ± 3.6	10 ± 4.7	4.2 ± 3.5	5 ± 3.8	100	48	9.2 ± 11.7	12.1 ± 14.8	5.2 ± 6.3	7.3 ± 7.8
*Peucedanum cervaria**	100	43	1.3 ± 1.1	3.1 ± 2.9	0	0	100	80	2.7 ± 1.5	3 ± 2.7	0	0
Primula veris	-	-	-	-	-	-	100	85	0.4 ± 1.3	0.3 ± 0.7	0	0
Salvia nemorosa	100	39	0.8 ± 1.2	1.7 ± 2	0	0	100	38	0.2 ± 0.4	0	0	0
*Salvia pratensis**	100	13	0.2 ± 0.4	0.3 ± 0.9	0	0	100	34	5.4 ± 4.9	3.2 ± 2.8	0	0
*Salvia verticillata**	100	10	1.7 ± 1.9	0.1 ± 0.3	0.3 ± 0.5	0	100	39	2.6 ± 2.2	0.4 ± 0.7	0.3 ± 0.5	0
*Sanguisorba minor**	100	51	4.1 ± 3.6	6 ± 5.1	0.7 ± 0.9	0	100	94	2.3 ± 2.4	4 ± 3.4	0.8 ± 0.9	0
*Scabiosa ochroleuca**	100	48	2.1 ± 2.1	2.8 ± 2.9	0.2 ± 0.4	0	100	76	3.9 ± 3.8	2.3 ± 1.9	0.9 ± 1.4	0
Sesseli hypomarathrum	100	19	0.6 ± 1.3	0.6 ± 1.1	0	0	-	-	-	-		
Stachys recta	100	15	0.1 ± 0.3	0	0	0	-	-	-	-		
*Tanacetum corymbosum**	85	20	1.4 ± 1.4	3.1 ± 1.6	0	0	100	66	5.8 ± 4.2	8.2 ± 7.5	0	0
*Teucrium chamaedrys**	100	64	2.2 ± 2	5.6 ± 3.4	0	0	100	67	2.1 ± 1.5	2.7 ± 1.3	0	0

Table 2. Effects of disturbance, species identity, locality and their interactions on proportion of established individuals, proportion of flowering individuals and maximum plant size per plot. Significant values are in bold. The proportion of established individuals is expressed as number of established individuals divided by number of sown viable seeds. The size of each plant was assessed as number of leaves × length of the longest leaf to approximate plant biomass (for details, see methods). The proportion of flowering individuals is expressed as number of flowering individuals divided by number of established individuals.

			Census 1			Census 2		
Response variable	Term	Df	R^2	quasi F	*P*	R^2	quasi F	*P*
Established individuals sown 2007	DISTurbance	1	**0.04**	**9.34**	**0.004**	**0.07**	**16.06**	**<0.001**
	SPECies	17	**0.22**	**7.74**	**<0.001**	**0.24**	**8.29**	**<0.001**
	LOCality	2	**0.04**	**4.71**	**0.024**	**0.05**	**7.77**	**0.004**
	DIST×SPEC	17	**0.07**	**2.47**	**0.001**	**0.05**	**1.72**	**0.041**
	DIST×LOC	2	0.01	2.59	0.090	**0.01**	**3.73**	**0.034**
	SPEC×LOC	34	**0.16**	**2.80**	**<0.001**	**0.15**	**2.56**	**<0.001**
	DIST×SPEC×LOC	34	**0.09**	**1.49**	**0.048**	0.07	1.19	0.228
	RESIDUAL	216	0.37			0.36		
Established individuals sown 2008	DISTurbance	1	0.00	0.15	0.704	0.00	0.76	0.390
	SPECies	17	**0.34**	**19.93**	**<0.001**	**0.29**	**12.62**	**<0.001**
	LOCality	2	**0.20**	**27.69**	**<0.001**	**0.19**	**28.25**	**<0.001**
	DIST×SPEC	17	**0.06**	**3.56**	**<0.001**	**0.06**	**2.46**	**0.001**
	DIST×LOC	2	0.01	2.85	0.072	0.01	1.70	0.199
	SPEC×LOC	34	**0.14**	**4.14**	**<0.001**	**0.11**	**2.48**	**<0.001**
	DIST×SPEC×LOC	34	0.05	1.47	0.053	0.06	1.28	0.153
	RESIDUAL	216	0.22			0.29		
Plant size sown 2008	DISTurbance	1	**0.13**	**65.68**	**<0.001**	**0.10**	**42.09**	**<0.001**
	SPECies	16	**0.30**	**14.88**	**<0.001**	**0.34**	**14.16**	**<0.001**
	LOCality	2	**0.09**	**18.47**	**<0.001**	**0.03**	**7.48**	**<0.001**
	DIST×SPEC	17	**0.04**	**2.11**	**0.008**	0.04	1.45	0.115
	DIST×LOC	2	**0.07**	**30.51**	**<0.001**	**0.02**	**4.85**	**0.014**
	SPEC×LOC	34	**0.07**	**1.60**	**0.025**	**0.08**	**1.68**	**0.015**
	DIST×SPEC×LOC	34	0.04	0.96	0.542	**0.08**	**1.68**	**0.015**
	RESIDUAL	216	0.26			0.31		
Flowering individuals sown 2008	DISTurbance	1				**0.36**	**360.98**	**<0.001**
	SPECies	17				**0.42**	**14.72**	**<0.001**
	LOCality	2				0.01	2.30	0.131
	DIST×SPEC	17				0.04	1.29	0.208
	DIST×LOC	2				**0.02**	**74.37**	**<0.001**
	SPEC×LOC	33				0.03	0.59	0.961
	DIST×SPEC×LOC	31				0.00	0.10	0.998
	RESIDUAL	152				0.25		

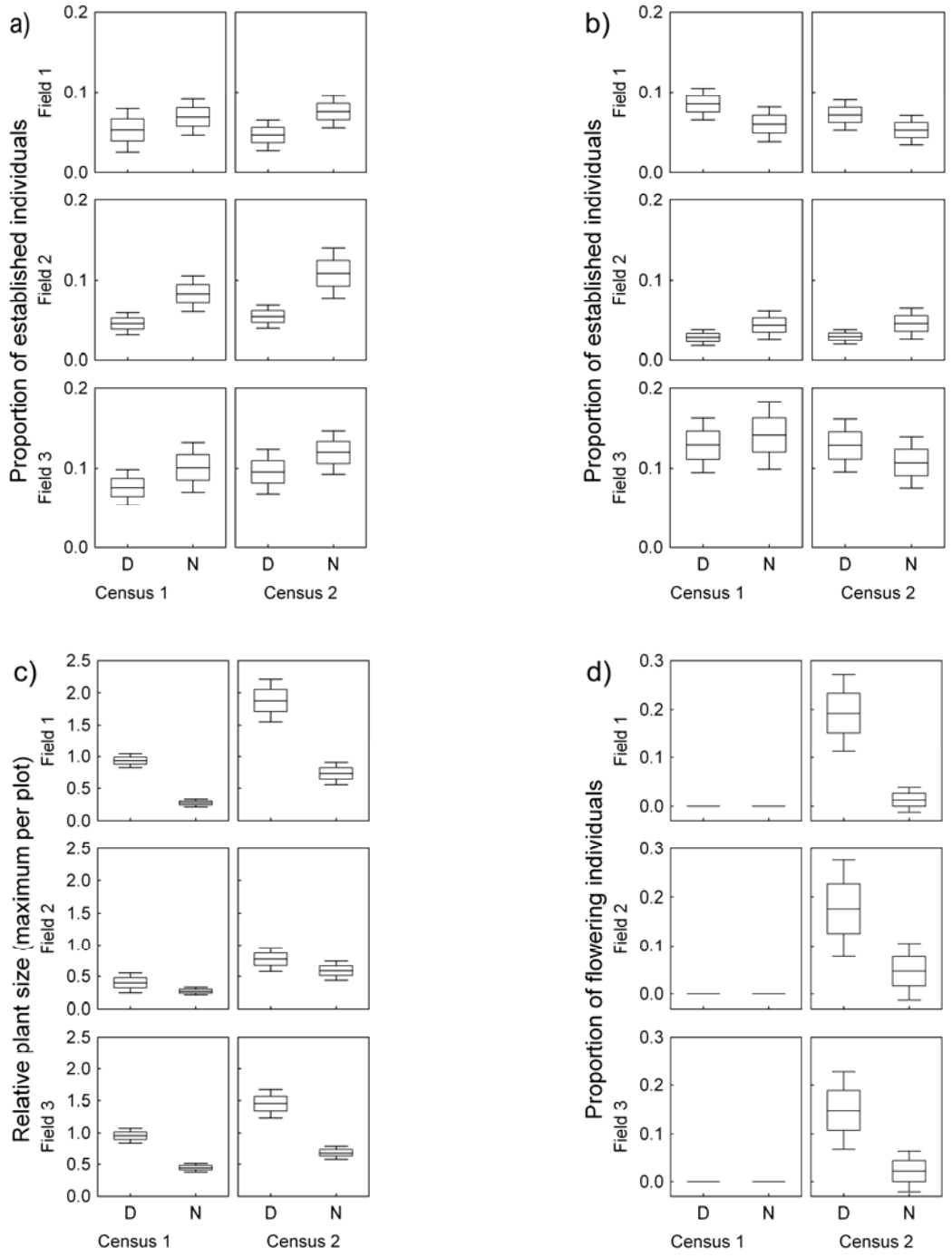
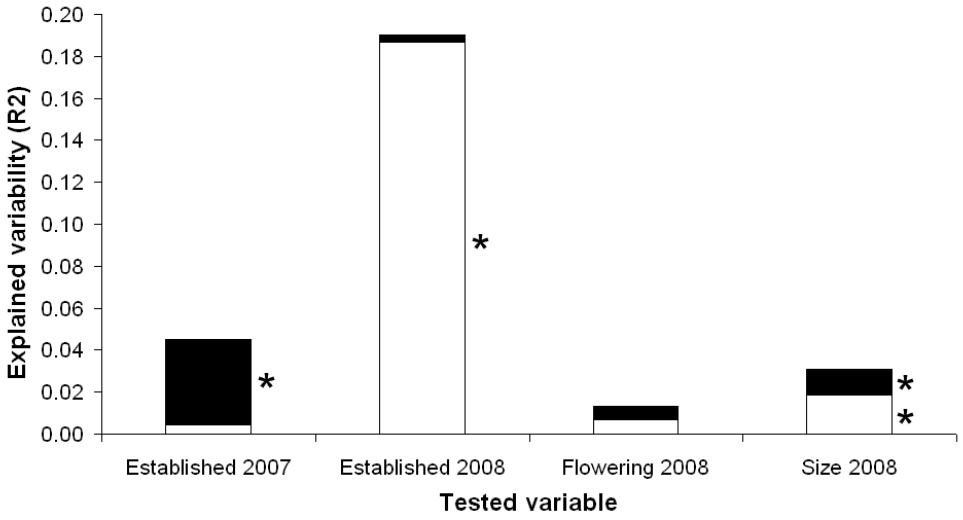
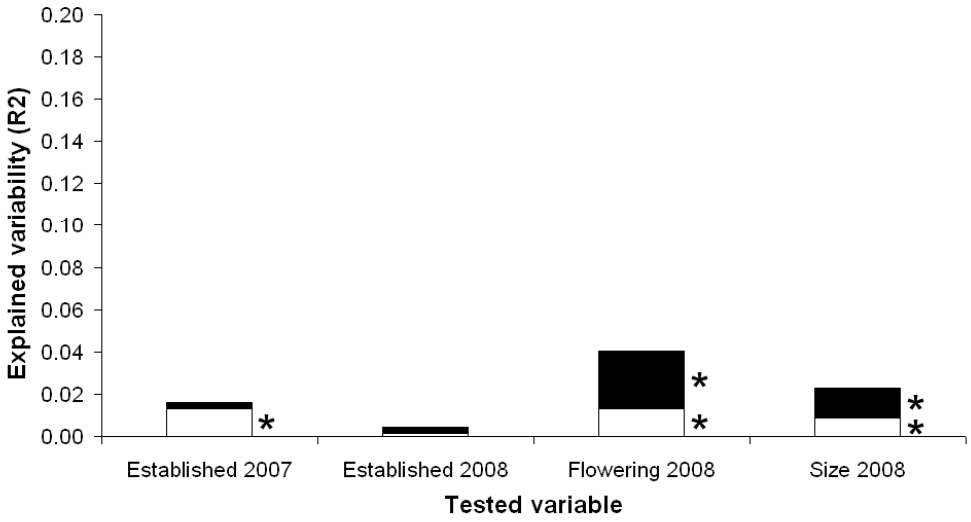


Fig. 1. Effect of disturbance on a) Proportion of established individuals sown in 2007. b) Proportion of established individuals sown in 2008. c) Relative size of plants sown in 2008. d) Proportion of flowering individuals sown in 2008. The proportion of established individuals is expressed as number of established individuals divided by number of sown viable seeds. Relative plant size is represented by the maximum size per plot divided by mean size of particular species across all plots. The size of each plant was assigned as number of leaves × length of the longest leaf to approximate plant biomass (for details, see methods). The proportion of flowering individuals is expressed as number of flowering individuals divided by number of established individuals. D – disturbed plots, N – undisturbed plots. Boxes show the mean ± SE, whiskers ± 1.96 SE.

Locality



Disturbance*Locality



Species*Locality

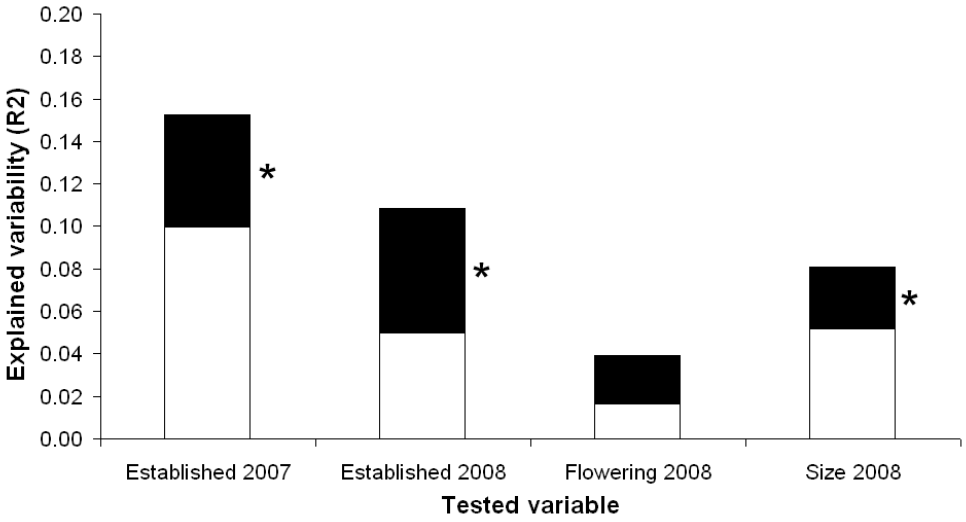


Fig. 2. Variability explained by locality, disturbance*locality and species*locality partitioned according to relative importance of soil parameters summarized in PCA. Asterisks are shown when the effect of PCA sample score of localities significantly affected plant performance (expressed either by proportion of established individuals, proportion of flowering individuals or plant size). PCA1 (black bars) is mostly correlated with P, C, N, K and Mg concentrations; PCA2 (white bars) is mostly correlated with WHC and pH (Appendix S2). Data are shown for second census. Note that the data originate from three localities and the two PCA axes thus explained all the variation which was explained by locality.

References

Bakker, J.D., Wilson, S.D., Christian, J.M., Li, X.D., Ambrose, L.G. & Waddington, J. (2003) Contingency of grassland restoration on year, site, and competition from introduced grasses. *Ecological Applications*, **13**(1), 137-53.

Bartha, S., Meiners, S.J., Pickett, S.T.A. & Cadenasso, M.L. (2003) Plant colonization windows in a mesic old field succession. *Applied Vegetation Science*, **6**(2), 205-12.

Bonser, S.P. & Reader, R.J. (1998) Species and biomass dependence of an indirect effect of vegetation on seedling recruitment. *Ecoscience*, **5**(2), 207-12.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P., Tielborger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B. & Michalet, R. (2008) Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology*, **96**(1), 18-34.

Burke, M.J.W. & Grime, J.P. (1996) An experimental study of plant community invasibility. *Ecology*, **77**(3), 776-90.

Connell, J.H. & Slatyer, R.O. (1977) Mechanisms of Succession in Natural Communities and Their Role in Community Stability and Organization. *American Naturalist*, **111**(982), 1119-44.

Cottrell, H.J. (1947) Tetrazolium Salt as a Seed Germination Indicator. *Nature*, **159**(4048), 748-48.

Cousins, S.A.O. (2009) Landscape history and soil properties affect grassland decline and plant species richness in rural landscapes. *Biological Conservation*, **142**(11), 2752-58.

Cramer, V.A., Hobbs, R.J. & Standish, R.J. (2008) What's new about old fileds? Land abandonment and ecosystem assembly. *Trends in Ecology & Evolution*, **23**(2), 104-12.

DeSimone, S.A. & Zedler, P.H. (1999) Shrub seedling recruitment in unburned Californian coastal sage scrub and adjacent grassland. *Ecology*, **80**(6), 2018-32.

Ehrlén, J., Münzbergová, Z., Diekmann, M. & Eriksson, O. (2006) Long-term assessment of seed limitation in plants: results from an 11-year experiment. *Journal of Ecology*, **94**(6), 1224-32.

Ellenberg, H., Weber, H., Düll, R., Wirth, V., Werner, W. & Paulissen, D. (1992) *Zeigerwerte der Gefassplanzen Mitteleuropas*, 3nd edn. Erich Goltze KG, Gottingen.

Eriksson, A. & Eriksson, O. (1997) Seedling recruitment in semi-natural pastures: the effects of disturbance, seed size, phenology and seed bank. *Nordic Journal of Botany*, **17**(5), 469-82.

Eriksson, O., Cousins, S.A.O. & Bruun, H.H. (2002) Land-use history and fragmentation of traditionally managed grasslands in Scandinavia. *Journal of Vegetation Science*, **13**(5), 743-48.

Fagan, K.C., Pywell, R.F., Bullock, J.M. & Marrs, R.H. (2008) Do restored calcareous grasslands on former arable fields resemble ancient targets? The effect of time, methods and environment on outcomes. *Journal of Applied Ecology*, **45**(4), 1293-303.

Fayolle, A., Violle, C. & Navas, M.L. (2009) Differential impacts of plant interactions on herbaceous species recruitment: disentangling factors controlling emergence, survival and growth of seedlings. *Oecologia*, **159**(4), 817-25.

Francis, B., Green, M., Payne, C.D. & Swan, T. (1993) *GLIM 4 : the statistical system for generalized linear interactive modelling* Clarendon Press, Oxford.

Goldberg, D.E. & Landa, K. (1991) Competitive Effect and Response - Hierarchies and Correlated Traits in the Early Stages of Competition. *Journal of Ecology*, **79**(4), 1013-30.

Gomez-Aparicio, L. (2009) The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. *Journal of Ecology*, **97**(6), 1202-14.

Gustavsson, E., Lennartsson, T. & Emanuelsson, M. (2007) Land use more than 200 years ago explains current grassland plant diversity in a Swedish agricultural landscape. *Biological Conservation*, **138**(1-2), 47-59.

Hellström, K., Huhta, A.P., Rautio, P. & Tuomi, J. (2009) Seed introduction and gap creation facilitate restoration of meadow species richness. *Journal for Nature Conservation*, **17**(4), 236-44.

Helm, A., Hanski, I. & Partel, M. (2006) Slow response of plant species richness to habitat loss and fragmentation. *Ecology Letters*, **9**(1), 72-77.

Hofmann, M. & Isselstein, J. (2004) Seedling recruitment on agriculturally improved mesic grassland: the influence of disturbance and management schemes. *Applied Vegetation Science*, **7**(2), 193-200.
Holmgren, M. & Scheffer, M. (2010) Strong facilitation in mild environments: the stress gradient hypothesis revisited. *Journal of Ecology*, **98**(6), 1269-75.
Howard, T.G. & Goldberg, D.E. (2001) Competitive response hierarchies for germination, growth, and survival and their influence on abundance. *Ecology*, **82**(4), 979-90.
Jakobsson, A. & Eriksson, O. (2000) A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. *Oikos*, **88**(3), 494-502.
Janssens, F., Peeters, A., Tallowin, J.R.B., Bakker, J.P., Bekker, R.M., Fillat, F. & Oomes, M.J.M. (1998) Relationship between soil chemical factors and grassland diversity. *Plant and Soil*, **202**(1), 69-78.
Jutila, H.M. & Grace, J.B. (2002) Effects of disturbance on germination and seedling establishment in a coastal prairie grassland: a test of the competitive release hypothesis. *Journal of Ecology*, **90**(2), 291-302.
Kahmen, S. & Poschlod, P. (2004) Plant functional trait responses to grassland succession over 25 years. *Journal of Vegetation Science*, **15**(1), 21-32.
Kleijn, D. (2003) Can establishment characteristics explain the poor colonization success of late successional grassland species on ex-arable land? *Restoration Ecology*, **11**(2), 131-38.
Knappová, J., Hemrová, L. & Münzbergová, Z. (2012) Colonization of central European abandoned fields by dry grassland species depends on the species richness of the source habitats: a new approach for measuring habitat isolation. *Landscape Ecology*, **27**(1), 97-108.
Kosola, K.R. & Gross, K.L. (1999) Resource competition and suppression of plants colonizing early successional old fields. *Oecologia*, **118**(1), 69-75.
Lanta, V., Klimešová, J., Martincová, K., Janeček, Š., Doležal, J., Rosenthal, J., Lepš, J. & Klimeš, L. (2011) A test of the explanatory power of plant functional traits on the individual and population levels. *Perspectives in Plant Ecology Evolution and Systematics*, **13**(3), 189-99.
Lavorel, S., Rochette, C. & Lebreton, J.D. (1999) Functional groups for response to disturbance in Mediterranean old fields. *Oikos*, **84**(3), 480-98.
Lepš, J. & Šmilauer, P. (2003) *Multivariate analysis of ecological data using CANOCO* Cambridge University Press, Cambridge.
Loehle, C. (2007) Effect of ephemeral stepping stones on metapopulations on fragmented landscapes. *Ecological Complexity*, **4**(1-2), 42-47.
Luoto, M., Rekolainen, S., Aakkula, J. & Pykala, J. (2003) Loss of plant species richness and habitat connectivity in grasslands associated with agricultural change in Finland. *Ambio*, **32**(7), 447-52.
McLellan, A.J., Law, R. & Fitter, A.H. (1997) Response of calcareous grassland plant species to diffuse competition: results from a removal experiment. *Journal of Ecology*, **85**(4), 479-90.
Milbau, A., Nijs, I., Van Peer, L., Reheul, D. & De Cauwer, B. (2003) Disentangling invasiveness and invasibility during invasion in synthesized grassland communities. *New Phytologist*, **159**(3), 657-67.
Münzbergová, Z. & Herben, T. (2005) Seed, dispersal, microsite, habitat and recruitment limitation: identification of terms and concepts in studies of limitations. *Oecologia*, **145**(1), 1-8.
Münzbergová, Z. (2004) Effect of spatial scale on factors limiting species distributions in dry grassland fragments. *Journal of Ecology*, **92**(5), 854-67.
Olff, H. & Ritchie, M.E. (1998) Effects of herbivores on grassland plant diversity. *Trends in Ecology & Evolution*, **13**(7), 261-65.
Osbornová, J., Kovářová, M., Lepš, J., Prach, K. & [eds.]. (1990) *Succession in abandoned fields: studies in central Bohemia, Czechoslovakia* Kluwer Academic, Dordrecht ; London.
Öster, M., Ask, K., Cousins, S.A.O. & Eriksson, O. (2009a) Dispersal and establishment limitation reduces the potential for successful restoration of semi-natural grassland communities on former arable fields. *Journal of Applied Ecology*, **46**(6), 1266-74.
Öster, M., Ask, K., Römermann, C., Tackenberg, O. & Eriksson, O. (2009b) Plant colonization of ex-arable fields from adjacent species-rich grasslands: The importance of dispersal vs. recruitment ability. *Agriculture Ecosystems & Environment*, **130**(3-4), 93-99.

Pakeman, R.J., Pywell, R.F. & Wells, T.C.E. (2002) Species spread and persistence: implications for experimental design and habitat re-creation. *Applied Vegetation Science*, **5**(1), 75-86.
Pánková, H., Münzbergová, Z., Rydlová, J. & Vosátka, M. (2008) Differences in AM fungal root colonization between populations of perennial Aster species have genetic reasons. *Oecologia*, **157**(2), 211-20.
Pywell, R.F., Bullock, J.M., Walker, K.J., Coulson, S.J., Gregory, S.J. & Stevenson, M.J. (2004) Facilitating grassland diversification using the hemiparasitic plant Rhinanthus minor. *Journal of Applied Ecology*, **41**(5), 880-87.
R Development Core Team. (2010). R: A language and environment for statistical computing. In. R Foundation for Statistical Computing, Vienna, Austria.
Reader, R.J. (1993) Control of Seedling Emergence by Ground Cover and Seed Predation in Relation to Seed Size for Some Old-Field Species. *Journal of Ecology*, **81**(1), 169-75.
Roberts, R.E., Clark, D.L. & Wilson, M.V. (2010) Traits, neighbors, and species performance in prairie restoration. *Applied Vegetation Science*, **13**(3), 270-79.
Ruprecht, E. (2006) Successfully recovered grassland: A promising example from Romanian old-fields. *Restoration Ecology*, **14**(3), 473-80.
Schupp, E.W. (1995) Seed Seedling Conflicts, Habitat Choice, and Patterns of Plant Recruitment. *American Journal of Botany*, **82**(3), 399-409.
Seabloom, E.W. (2011) Spatial and temporal variability in propagule limitation of California native grasses. *Oikos*, **120**(2), 291-301.
Špačková, I. & Lepš, J. (2004) Variability of seedling recruitment under dominant, moss, and litter removal over four years. *Folia Geobotanica*, **39**(1), 41-55.
Steffan-Dewenter, I. & Tscharntke, T. (1997) Early succession of butterfly and plant communities on set-aside fields. *Oecologia*, **109**(2), 294-302.
Ter Braak, C.J.F. & Šmilauer, P. (2002). CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). In. Microcomputer Power, Ithaca, NY, USA.
Tilman, D. (1997) Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology*, **78**(1), 81-92.
Turnbull, L.A., Crawley, M.J. & Rees, M. (2000) Are plant populations seed-limited? A review of seed sowing experiments. *Oikos*, **88**(2), 225-38.
Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM and Webb DA, Editors. 1964-1980. *Flora Europaea* **Vols. 1–5**, Cambridge: Cambridge University Press.
Vaughn, K.J. & Young, T.P. (2010) Contingent Conclusions: Year of Initiation Influences Ecological Field Experiments, but Temporal Replication is Rare. *Restoration Ecology*, **18**, 59-64.
Veblen, K.E. (2008) Season- and herbivore-dependent competition and facilitation in a semiarid savanna. *Ecology*, **89**(6), 1532-40.
Vítová, A. & Lepš, J. (2011) Experimental assessment of dispersal and habitat limitation in an oligotrophic wet meadow. *Plant Ecology*, **212**(8), 1231-42.
Walker, K.J., Stevens, P.A., Stevens, D.P., Mountford, J.O., Manchester, S.J. & Pywell, R.F. (2004) The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation*, **119**(1), 1-18.
Web1. http://portal.chmi.cz/ downloaded 26 April 2011
Westoby, M. (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil*, **199**(2), 213-27.
Westoby, M., Leishman, M. & Lord, J. (1996) Comparative ecology of seed size and dispersal. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **351**(1345), 1309-17.
Zeiter, M., Stampfli, A. & Newbery, D.M. (2006) Recruitment limitation constrains local species richness and productivity in dry grassland. *Ecology*, **87**(4), 942-51.

SUPPORTING INFORMATION

Appendix S1 *Weather of sowing years.*

Appendix S2 *Characteristics of experimental fields.*

Table S1 *Full model including interactions with sowing year.*

Appendix S1. Monthly sums of precipitation and means of temperature compared with the long-term normal 1961–1990 over the territory of the Usti nad Labem administrative region (Czech Republic).

Monthly sums of precipitation

		1	2	3	4	5	6	7	8	9	10	11	12	Sum
Long-term normal [mm]		42	36	38	44	61	68	68	70	50	39	47	49	612
2007	mm	67	52	34	2	96	69	86	102	89	26	84	25	732
	% of normal	161	143	89	5	157	101	127	147	178	66	181	51	120
2008	mm	48	27	47	68	36	61	71	70	39	69	35	46	616
	% of normal	114	76	124	154	58	89	104	101	77	176	75	95	101
2009	mm	21	50	62	21	100	77	95	61	23	68	45	58	681
	% of normal	51	139	164	48	162	113	140	88	45	172	96	119	111
2010	mm	46	20	39	27	93	49	128	188	105	12	83	92	879
	% of normal	110	55	103	60	152	72	188	268	210	30	176	187	144

Monthly means of air temperature

YEAR	Month	1	2	3	4	5	6	7	8	9	10	11	12	Year
Long-term normal [°C]		-2.4	-0.9	2.8	7.5	12.4	15.8	17.2	16.6	12.9	8.1	2.9	-0.6	7.7
2007	°C	3.9	3.2	5.5	10.9	14.7	18.2	18.2	17.6	11.8	7.2	2.1	0.0	9.4
	deviation from long-term normal	6.3	4.1	2.7	3.4	2.3	2.4	1.0	1.0	-1.1	-0.9	-0.8	0.6	1.7
2008	°C	1.7	3.4	3.5	7.7	13.8	17.4	18.0	17.4	11.9	8.0	4.3	0.7	9.0
	deviation from long-term normal	4.1	4.3	0.7	0.2	1.4	1.6	0.8	0.8	-1.0	-0.1	1.4	1.3	1.3
2009	°C	-3.7	-0.3	4.0	12.4	13.4	14.9	17.9	18.5	14.9	7.5	5.7	-1.2	8.7
	deviation from long-term normal	-1.3	0.6	1.2	4.9	1.0	-0.9	0.7	1.9	2.0	-0.6	2.8	-0.6	1.0
2010	°C	-4.7	-1.9	3.2	8.3	11.3	16.8	20.4	17.0	11.4	6.4	4.6	-5.3	7.3
	deviation from long-term normal	-2.3	-1.0	0.4	0.8	-1.1	1.0	3.2	0.4	-1.5	-1.7	1.7	-4.7	-0.4

source: http://portal.chmi.cz/ downloaded 26 April 2011

Appendix S2. Detailed description of the three experimental fields.

In the table, mean ± SD of soil parameters are given.
In PCA of soil nutrients, minerals and water holding capacity (see figure), soil parameters were used as species, localities (experimental fields) as independent nominal variables. Data were centered and standardized by species. First axis explained 74.4%, second axis explained 25.6% of variability in data. Effect of field was significant (P = 0.002) in MonteCarlo permutation test with 999 permutations and restricted spatial design. Multivariate analysis was performed in Canoco for Windows 4.5 (Ter Braak et Šmilauer 1998).

	Field 1	Field 2	Field 3
Longitude	14°19'32.651"E	14°13'26.244"E	14°13'36.828"E
Latitude	50°31'35.444"N	50°31'43.733"N	50°31'39.465"N
Dominant species	*Arrhenatherum elatius* *Dactylis glomerata*	*Daucus carota* *Cirsium arvense*	*Elytrigia intermedia*
Soil water holding capacity (WHC)	0.29 ± 0.02	0.31 ± 0.03	0.34 ± 0.03
pH(H2O)	7.96 ± 0.08	7.99 ± 0.06	7.82 ± 0.1
pH(KCl)	7.65 ± 0.06	7.59 ± 0.04	7.5 ± 0.04
N [%]	0.12 ± 0.02	0.17 ± 0.02	0.15 ± 0.02
C-total [%]	3.68 ± 0.35	8.54 ± 0.23	7.82 ± 0.22
C-carbon [%]	2.19 ± 0.1	6.62 ± 0.26	6.06 ± 0.21
C-organic [%]	1.49 ± 0.35	1.92 ± 0.29	1.76 ± 0.25
Ca [mg/1000g]	23718 ± 3390	32079 ± 2526	34634 ± 10062
Mg [mg/1000g]	119 ± 24	180 ± 16	146 ± 20
K [mg/1000g]	116 ± 34	479 ± 56	284 ± 56
P [mg/1000g]	10.3 ± 2.6	6.5 ± 0.9	7.3 ± 1.6

Table S1. Effect of disturbance, species identity, sowing year and locality on the proportion of individuals recorded in sowing squares related to the numbers of sown viable seeds. Significant values are in bold.

			Census 1			Census 2		
Term	Df	Error Term	R2	quasi F	*P*	R2	quasi F	*P*
DISTurbance	1	SPEC:YEAR:LOC	**0.01**	**5.56**	**0.024**	**0.01**	**6.41**	**0.016**
SPECies	17	RESIDUAL	**0.22**	**20.98**	**<0.001**	**0.19**	**15.02**	**<0.001**
Sowing YEAR	1	DIST:SPEC:LOC	0.00	0.41	0.524	**0.01**	**10.06**	**0.003**
LOCality	2	DIST:SPEC:YEAR	**0.09**	**63.74**	**<0.001**	**0.08**	**82.48**	**<0.001**
DIST:SPEC	17	RESIDUAL	**0.05**	**4.53**	**<0.001**	**0.04**	**3.57**	**<0.001**
DIST:YEAR	1	DIST:SPEC:YEAR:LOC	**0.00**	**8.01**	**0.008**	**0.02**	**31.84**	**<0.001**
DIST:LOC	2	DIST:SPEC:YEAR:LOC	0.00	1.98	0.153	**0.01**	**5.18**	**0.011**
SPEC:YEAR	17	RESIDUAL	**0.06**	**5.76**	**<0.001**	**0.06**	**5.20**	**<0.001**
SPEC:LOC	34	RESIDUAL	**0.09**	**4.28**	**<0.001**	**0.09**	**3.42**	**<0.001**
YEAR:LOC	2	DIST:SPEC:YEAR:LOC	**0.03**	**31.62**	**<0.001**	**0.03**	**25.86**	**<0.001**
DIST:SPEC:YEAR	17	RESIDUAL	0.01	1.15	0.308	0.01	0.66	0.840
DIST:SPEC:LOC	34	RESIDUAL	**0.05**	**2.13**	**<0.001**	**0.04**	**1.68**	**0.011**
DIST:YEAR:LOC	2	DIST:SPEC:YEAR:LOC	**0.01**	**7.48**	**0.002**	**0.00**	**3.28**	**0.049**
SPEC:YEAR:LOC	34	RESIDUAL	**0.06**	**2.65**	**<0.001**	**0.04**	**1.69**	**0.011**
DIST:SPEC:YEAR:LOC	34	RESIDUAL	0.02	0.83	0.736	0.02	0.79	0.795
RESIDUAL	432		0.27			0.32		

Quantity is more than quality: Abundance in the landscape rather than dispersal traits determines colonization success in grassland plants

Jana Knappová, Lucie Hemrová, Michal Knapp & Zuzana Münzbergová

submitted manuscript

Quantity is more than quality: Abundance in the landscape rather than dispersal traits determines colonization success in grassland plants

Jana Knappová[1,2], Lucie Hemrová[1,2], Michal Knapp[3], Zuzana Münzbergová[1,2]

[1]Department of Botany/Ecology, Faculty of Science, Charles University in Prague, Benátská 2, 128 01, Prague, Czech Republic
[2]Institute of Botany, Academy of Sciences of the Czech Republic, 252 43, Průhonice, Czech Republic
[3]Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, 165 21, Prague, Czech Republic

Abstract

Dispersal limitation, i.e. the lack of available seeds resulting in suitable habitats staying unoccupied, is reported in many plant species based on seed addition experiments. However, these experiments can not distinguish whether the failure of a species to colonize suitable habitat was due to its dispersal traits or due to low availability of seeds in the surrounding landscape.
In our study, colonization ability is expressed as proportion of occupied abandoned fields, which are thought to be potential habitats for grassland species. Three distinct types of limitation were represented by three groups of traits: availability of seeds in the surrounding landscape, dispersal traits and habitat requirements and their relative effect on species frequency in abandoned fields was assessed using variance partitioning. Both cross-species analysis and analysis of phylogenetically independent contrasts were performed leading to similar results.
We showed that many dry grassland species failed to reach abandoned fields not because of their poor dispersal traits but due to low availability of seeds in the surrounding landscape represented mainly by number of source populations and only slightly by seed production and length of flowering period. Therefore, dispersal *per se* might not be the main cause of dispersal limitation detected by amounts of seed addition experiments. Colonization ability was further influenced by habitat requirements, especially niche width. Some of habitat requirements or other hidden traits possibly cause species rarity and commonness in both source and target habitats. However, variation in colonization ability explained by availability of seeds and especially by frequency in source grasslands was so large that we expect some kind of positive feedback, independent of traits. Once a species become frequent in source habitats, it is also much likely to be frequent in target habitats, more than expected from its trait-driven colonization ability. Our results thus provide important empirical support that both neutral and niche processes are taking part in assembly of ecological communities.

Keywords: commonness, dispersal, species traits, distribution, habitat limitation, rarity, Neutral theory, seed addition, seed availability

Introduction

Understanding factors affecting species distribution in the landscape is fundamental challenge in ecology. With the ongoing habitat loss and fragmentation, it becomes even more important to identify factors affecting occurrence of species to ensure effective conservation of diversity at landscape scales. In particular, it is crucial to identify why species are missing from communities to separate effects of intrinsic species traits and extrinsic characteristics of biotic and abiotic environment.

The absence of individual species from a site can result either from unsuitable local conditions for a species to grow there (habitat limitation) or from the inability of species' seeds to reach the site in sufficient quantity (dispersal limitation; Münzbergová & Herben, 2005). However, dispersal limitation itself can have two different reasons. Plants may be limited either by their poor dispersal throughout the landscape or simply by the insufficient number and/or size of source populations resulting in the lack of seeds ready to disperse. While separating dispersal and habitat limitation is relatively common in studies involving diverse habitats (e.g., Ehrlén & Eriksson, 2000; Münzbergová, 2004; Öster et al., 2009), the differentiation between the two sources of dispersal limitation received almost no attention (but see Terborgh et al., 2011).

When dispersal limitation of a species is acknowledged, it is often thought to be related to dispersal parameters such as seed mass, terminal velocity, presence of dispersal structures, etc (Ehrlén & Eriksson, 2000; Tremlová & Münzbergová, 2007). Nonetheless, high seed production or high abundance in the landscape might compensate for otherwise low dispersal ability. It is therefore important to disentangle these factors when assessing why species are not present at some sites. Such knowledge is an essential baseline for any further conservation efforts (Murray et al., 2002; Heywood & Iriondo, 2003).

Semi-natural dry grasslands are among the most threatened habitats in Europe, endangered mainly by cessation of former management practices and conversion into other types of land use (Poschlod & Bonn, 1998; Cousins, 2009). To assure effective conservation of remaining grassland diversity, much work has been done to reveal factors explaining species occurrences in grassland fragments (e.g., Bruun, 2000; Tremlová & Münzbergová, 2007; Chýlová & Münzbergová, 2008). However, the very slow response of perennial plants to landscape changes often impedes to accurately evaluate why species occur just where they occur and not elsewhere (Cousins et al., 2007; Cousins, 2009).

We study the ongoing process of colonization of recently abandoned fields by species from current dry grasslands. A previous work in the same region demonstrated that some portions of the current area of dry grasslands were arable fields in the 1950's or even in 1980's (Chýlová & Münzbergová, 2008), suggesting that grassland species have been able to spread into novel habitats. Furthermore, many grassland species also already occur in recently abandoned fields (Knappová et al., 2012). We therefore assume that abandoned fields in this area represent potential habitats for dry grassland species.

Clear distinction between source and target habitats (grasslands and abandoned fields) in our study system provides an excellent opportunity to separate factors affecting species distribution from confounding effects of diverse history of land use at individual sites (see also Knappová et al., 2012). To our knowledge, decomposing dispersal limitation in two parts related either to species

dispersal ability or seed availability has never been done in herbs. Only few examples exist in trees (Gomez-Aparicio et al., 2007; Terborgh et al., 2011), where one could expect substantial differences in dispersal mechanisms as well as seed productivity and abundance patterns. Therefore, our main aim was to assess whether successful establishment of dry grassland species in the studied abandoned fields is limited by habitat requirements of species, species traits related to dispersal or by availability of seeds in the landscape. In particular, we are interested in the relative importance of the three above mentioned sources of limitation for species occurrences on abandoned fields.

Methods

Study region and target habitats

Data on species occurrences used in this study were collected in the northern part of the Czech Republic in an area approximately 8.5 by 8.5 km (for details, see Knappová et al., 2012). Natural vegetation in the region is represented by remnants of oak-hornbeam and thermophilous oak forests (alliance Carpinion and Quercion petrae; Ellenberg, 1988). Fields abandoned in the last 20 years are already overgrown with grasses and ruderal herbaceous vegetation, e.g., Arrhenatherum elatius, Dactylis glomerata, Cirsium arvense and Melilotus spp. Semi-natural calcareous dry grasslands (alliance Bromion erecti; Ellenberg, 1988) occur in small fragments and they host a vast small-scale diversity of vascular plants (Münzbergová, 2004; Chýlová & Münzbergová, 2008), including a number of threatened species. At present, most of the grasslands in the region are not managed, and occasionally, some of them are completely destroyed by human activities (e.g., ploughing or development of solar power plants). In total, 46 abandoned fields (target habitats) and 339 dry grasslands (source habitats) were included in the dataset.

Target species and data on species traits

The study involves 32 perennial plant species typically occurring in dry grasslands within the study region, comprising a large spectrum from very rare to almost ubiquitous ones (Table 1). For each species, we assembled information on its frequency in abandoned fields (target habitats) as a measure of colonization ability. Further, three distinct sources of limitation were represented by three groups of traits: availability of seeds in the surrounding landscape, seed dispersal traits and species habitat requirements (Table 2). For simplicity, we called all variables involved as traits although some of them do not match exact definition of a trait (e.g, they are not measured at individual level or independently on environmental conditions; Violle et al., 2007).

Availability of seeds (A)

For each species, we assessed three different indicators of seed availability in the landscape: frequency and seed production in source habitats (i.e., dry grasslands) and length of flowering period representing seed availability in time. Frequency in grasslands was assessed as proportion of all source grasslands (from total 339) where the species was present (Knappová et al., 2012). Seed production at landscape level was assessed by multiplying mean field seed production and seed viability. Field seed production was estimated in three populations of each of target species in the studied region. For each population the mean number of

developed seeds per plant was assessed using 20 randomly selected individuals. This number was multiplied by the mean number of flowering shoots per 1 m2, which was counted in five selected quadrates within a population of the species at the three localities. The quadrates were located in places with as high density of the focal species as possible to capture maximum density the plants can achieve in the field. Part of the data on mean field seed production comes from Tremlová & Münzbergová (2007). Seed viability was assessed by tetrazolium test (Cottrell, 1947) using three groups of 100 seeds. Values of seed viability were used also in calculation of field germinability and field competitiveness (see bellow). Field seed production was log transformed prior analyses. Length of flowering period was excerpted from Key to the Flora of the Czech Republic (Kubát et al., 2002).

Seed dispersal traits (D)

Information on seed weight, dispersal distance, attachment ability and rate of endozoochory was assembled for each species (Tremlová & Münzbergová, 2007, D. Průchová, unpublished data). Seed weight was estimated by weighing five groups of 10 seeds from three source populations (150 seeds in total). Seed weight was log transformed prior analyses. Dispersal distance (D) was calculated using the simple formula (Soons & Heil, 2002) D = w*h / t, where w is wind speed (m.s-1), h is the release height (m) and t is terminal velocity (m.s-1) of the species. We used the maximum daily mean wind speed over June and July, as detected by the Czech Hydrometeorological Institute at the nearby meteorological station in Doksany from 2005 to 2010 (9.6 m.s-1) as the wind speed w. Release height h (Kleyer et al., 2008) was measured for ten randomly chosen individuals within three populations (30 individuals in total). Terminal velocity t was measured using dropping method (Jongejans & Schippers, 1999). Ten seeds from three populations (30 seeds in total) were released from 2 m height; each seed was released three times. Attachment ability, used as an estimate of the ability to disperse via exozoochory, was assessed by gently placing a piece of sheep fur over a tray containing 100 seeds, removing it, shaking ten times and counting the number of attached seeds (Münzbergová, 2004). This procedure was repeated with four sets of seeds for each species (400 seeds in total) and mean value was then used for each species. Rate of endozoochory express the mean percentage seeds having survived simulated digestion in comparison to the control. The simulation of ingestion and digestion includes a mechanical treatment representing chewing and a chemical treatment standing for seed digestion in the abomasum (Kleyer et al., 2008). It was measured for each species using five sets of 150 seeds originating from three populations (2250 seeds in total).

Species habitat requirements (H)

Traits related to establishment on abandoned fields (field germinability and field competitiveness) were assessed for each species based on data from seed sowing experiment (J. Knappová unpublished data). The experiment investigated the establishment success following sowing of dry grassland species on eight abandoned fields within the same study region. At each field, 100 seeds per species (with a few exceptions) were sown in three plots with and three plots without vegetation removal (disturbed and undisturbed, respectively). The experiment was repeated in two consecutive years (2007 and 2008). Field germinability was assessed as total number of individuals of particular species present in the second year following sowing, expressed as proportion of sown

viable seeds. Field competitiveness was calculated as log response ratio (LRR; Hedges et al., 1999) as follows LRR = $\ln(N_{UNDISTURBED}/N_{DISTURBED})$ where N stands for total number of individuals established from total number of sown viable seeds in undisturbed and disturbed plots respectively. The higher absolute value of LRR, the larger difference between seedling numbers at disturbed and undisturbed plots, positive numbers denote more seedlings being established in undisturbed plots. In this way the resulting variable express the ability of species to establish under vegetation canopy (positive LRR values), or in other words, species preference of gaps or early successional stages (negative LRR values).

As an indirect evidence for species habitat requirements, we used data on Ellenberg indicator values (Ellenberg et al., 1992) for light, temperature, continentality, moisture, soil reaction and nutrients. The values are simple ordinal classes indicating optima of realized ecological niche along a gradient. The indicator values were developed mainly on the basis of field experience and reflect the ecological behaviour of species (Diekmann & Dupré, 1997).

Finally, estimate of niche width, θ, was assessed for each species using co-occurrence based approach (Fridley et al., 2007). All relevés containing at least one of 32 target species were selected from Czech National Phytosociological Database (Chytrý & Rafajová, 2003) resulting in 20 853 relevés in total. Because plot richness distributions among species were non-skewed in our dataset (data not shown), we could use the multiplicative Whittaker's beta diversity measure for calculation of θ (Manthey & Fridley, 2009; Zelený, 2009).

Phylogenetic information

Closely related species share many traits in addition to those under study and these hidden traits could be in fact responsible for ecological success in particular circumstances (Harvey, 1996). Moreover, due to sharing of common ancestors related species do not provide independent information for testing ecological hypotheses (Harvey et al., 1995). Therefore, phylogenetic information should be incorporated into trait analyses. Analysis of data within a phylogenetic framework can also reveal patterns of association of species traits that would be masked by simple across-species comparisons (Harvey, 1996).

Phylogenetic tree (Figure S1 in Supporting Information) was constructed using online Phylomatic software (Webb & Donoghue, 2005) and it was completely resolved based on additional information (Web 1). Phylogenetically independent contrasts (PICs; Felsenstein, 1985) were subsequently calculated with function crunch in R package 'caper' (Orme et al., 2012). The method of independent contrasts uses phylogenetic information to transform interspecific data into values that, in principle, are independent and identically distributed, and hence can be analyzed with standard statistical methods such as regression. Algorithm implemented in crunch calculates standardized PICs according to Pagel (1992).

We are aware that presentation of both results from cross-species analysis and analysis of PICs has been criticised due to their different assumptions on data distribution (Freckleton et al., 2002; Freckleton, 2009). Recently, several methods have been proposed to select the most proper model of trait evolution and to test and correct for phylogenetic signal in comparative data (Pagel, 1999; Freckleton et al., 2002; Revell, 2010). More specifically, such methods determine the degree to which the variation in particular trait is related to phylogeny (Pagel's λ) and allow adjusting further analysis appropriately. Application of such method,

however, is complicated in case of multiple regression as individual traits naturally differ in this degree of phylogenetic dependence (Pocock et al., 2006). So far, estimating whole model λ by maximum likelihood is possible only in linear regression (Revell, 2010) whereas we use response variable with binomial distribution of errors. Therefore, we decided to follow more traditional approach with PICs.

In our study, most traits exhibited very low degree of phylogenetic dependence (Appendix S1 in Supporting Information). Simple cross-species (uncorrected) analyses were suggested to be more appropriate than conventional phylogenetic approaches for data including both traits that show phylogenetic dependence and others that do not (Pocock et al., 2006). We therefore use analyses with PICs mainly to correct for possible confounding effects of hidden traits (i.e. those not involved in the study; Harvey et al., 1995; Harvey, 1996).

Data analysis

For the analyses of effect of traits on species colonization ability, proportion of occupied fields (from total 46) was used as dependent variable and species traits representing the three types of limitation (Table 2) as independent variables. We used generalised linear models with a quasi-binomial distribution to analyze species data and linear models to analyze PICs (hence PICs are transformed to be normally distributed). At the first step, we selected best model using forward selection based on F-tests. Selection was performed independently within each group of traits (availability of seeds, dispersal traits, habitat requirements). Applying the selection to all traits combined could result in the elimination of some variables from one trait group because they are correlated with variables in the other trait group (Legendre & Legendre, 1998). Model selection was repeated with the PICs instead of species.

In the second step, we applied variance partitioning to evaluate variance explained by the three potential sources of limitation (availability of seeds, dispersal traits, and habitat requirements). Variance partitioning was applied to both full and best models based either on species or PICs as items of evidence.

All analyses were performed in R 2.14.1 (R Development Core Team, 2011).

Results

Eight out of 32 target species were not found on any of the abandoned fields within study region, while 11 species occurred on more than half of the fields (Table 1). Only a few traits involved were correlated among each other and many correlations disappeared after phylogenetic correction (Table S1 in Supporting Information).

According to variance partitioning of the full model, species frequency in abandoned fields was affected mainly by availability of seeds (i.e., frequency in source grasslands, seed production and length of flowering period). The net effect of species dispersal traits and habitat requirements was lower than net effect of availability of seeds almost four and three times respectively (Fig. 1a). In the best model, nearly three quarters of variability in species frequency in abandoned fields was explained by species abundance in source grasslands and seed production (Fig. 2a,e), 18% of which was shared with the effect of niche width (Fig. 1c, 2c). Niche width alone had only negligible net effect and none of the

dispersal traits had significant effect on frequency in abandoned fields (Table 3, Fig. 1c).

The relative importance of the three types of limitation based on the full model changed only slightly after phylogenetic correction (Fig. 1b). In the best model, the net effect of seed availability on species frequency in abandoned fields decreased on behalf of shared effect with habitat requirements, namely niche width and EIV for moisture (Table 3, Fig. 1d, 2b,d,f), whereas the overall effect remain unchanged. Dispersal traits had no significant effect (Table 3, Fig. 1d).

Discussion

Dispersal limitation, i.e. the lack of available seeds resulting in suitable habitats staying unoccupied, is reported in many plant species (Münzbergová, 2004; Öster et al., 2009; Vítová & Lepš, 2011). The most direct approach to identify suitable unoccupied habitats is by means of seed addition experiments (Turnbull et al., 2000). However, seed addition experiments can not distinguish whether the failure of a species to colonize suitable habitat was due to its dispersal traits or due to low availability of seeds in the surrounding landscape.

In our study, colonization ability is expressed as frequency of species in abandoned fields, which are thought to be potential habitat for grassland species. Separating of source and target habitats allowed for distinction between limitation by dispersal ability and limitation by availability of seeds. One can argue that populations established in abandoned fields could also serve as seed sources and that our distinction is arbitrary. Populations of grassland species on abandoned fields, however, are rather small compared to populations in grasslands and in a previous study, we showed that their importance as sources of seeds is negligible (Knappová et al., 2012).

In present study, we demonstrate that the most limiting factor for successful colonization is availability of seeds which is given mainly by the number of source populations in grasslands. In contrast to other studies (e.g., Tremlová & Münzbergová, 2007; Öster et al., 2009), dispersal traits seem to be of minor importance for colonization ability in this system. We did not find much the same evidence in current literature though almost no studies differentiating the two sources of dispersal limitation exist. To our knowledge, only Ruprecht (2006) made similar remark in grasslands, but she did not test explicitly the relative importance of different types of limitation. At smaller spatial scale, Uriarte et al. (2010) also showed that seed production in a forest herb was much more limiting than seed dispersal, while habitat limitation had no significant effect on establishment. Our results thus bring important finding that the role of dispersal per se might not be the main cause of dispersal limitation detected by amounts of seed addition experiments.

To comprehensively express the three types of limitation, we employed heterogeneous group of variables. Some of them represent landscape-level parameters or performance measures assessed for individual species rather than being plant traits in strict sense (Violle et al., 2007). One the other hand, these variables might be manifestation of other functional or performance trait(s). This in turn rises a problem that what we reported as being causal relationship might be in fact of correlative nature between dependent and independent variables (Freckleton, 2009). A species might be abundant in abandoned fields not because it is abundant in grasslands but because abundance in fields as well as abundance

in grasslands is given by other important trait. Indeed, we have shown that quite high amount of variance in colonization ability was attributable to the shared effect of habitat requirements (especially niche width) and availability of seeds (frequency in grasslands).

In the best model, including phylogenetic information into analyses considerably decreased net effect of frequency in grasslands on behalf of shared effect with habitat requirements (niche width and EIV for moisture, cf. Fig. 1c,d). Though confounding effect of hidden traits is likely to be reduced by phylogenetic correction (Harvey, 1996), this further implies that higher frequency in both grasslands and abandoned fields is associated with wider niche or alternatively with hidden traits responsible for wider niche. For example, in the same study system of dry grasslands, Münzbergová (2005) found low local population growth rates to be responsible for species rarity at landscape scale. Similarly, difference in growth rate between rare and common species was shown in meta-analysis within Angiosperms (Murray et al., 2002). However, as slower growth is thought to be typical for species of lower competitive ability, later successional stages or nutrient poor habitats (Gleeson & Tilman, 1994; Aerts, 1999), we are confident that other traits involved in the study (e.g., field competitiveness, EIV for nutrients, niche width) cover to a large extent also for differences in species growth rate.

Although habitat requirements, niche width or other hidden traits could partly account for species commonness in both source and target habitats, we still found a large portion of variation in colonization ability being explained solely by availability of seeds and especially by frequency in source grasslands. It is unlikely that despite a wide range of traits under study there are still some very influential and phylogenetically independent hidden traits that determine species abundance in both source and target habitats. Rather, this tight relationship between species frequency in dry grasslands and in abandoned fields implies some kind of positive feedback. Once a species become frequent in source habitats, it is also much likely to be frequent in target habitats, more than expected from its trait-driven colonization ability. Such positive feedback does not need to apply only in case of colonization of novel habitats, but likely also on distribution patterns in existing grasslands. Being solely a result of neutral mechanisms, rare species are more extinction-prone, and once they go locally extinct, they take longer to re-immigrate than do common species (Volkov et al., 2003). Our results thus provide important empirical support that both neutral and niche processes are taking part in assembly of ecological communities.

Conclusion

Species ability to disperse from one site to another attracts attention of ecologist especially with the ongoing landscape changes. Traits enhancing dispersal are thought to be of vital importance when habitats are becoming more fragmented and isolated. We showed that many dry grassland species fail to reach potentially suitable abandoned fields not because of their poor dispersal traits but due to low availability of seeds in the surrounding landscape. The latter is given mainly by number of source populations and only slightly by seed production and length of flowering period. Some of habitat requirements (e.g., niche width) or other hidden traits possibly cause species rarity and commonness in both source and target habitats. However, variation in colonization ability explained by

availability of seeds and especially by frequency in source grasslands was so large that we expect some kind of positive feedback, independent of traits.

Acknowledgments

We thank to D. Průchová for providing data on species traits. Work of J.K., L.H. and Z.M. was supported by the grant Agency of Czech Republic (Grants no. P505/10/0593 and P504/10/0456), and partly by project no. RVO 67985939. M.K. was funded by grant IGA no. 42110/1312/3124.

Table 1. List of target dry grassland species. Number of occupied abandoned fields (from total 46) and of occupied grasslands (from total 339) is shown. Nomenclature follows Kubát *et al.* (2002)

Species	Fields	Grasslands
Agrimonia eupatoria	44	311
Anthericum ramosum	0	27
Aster amellus	2	29
Astragalus cicer	24	119
Astragalus glyciphyllos	33	199
Brachypodium pinnatum	27	286
Bromus erectus	10	135
Bupleurum falcatum	30	219
Carex flacca	4	137
Carex tomentosa	4	76
Carlina vulgaris	13	85
Centaurea jacea	33	238
Centaurea scabiosa	22	222
Coronilla vaginalis	0	4
Filipendula vulgaris	0	17
Globularia bisnagarica	0	16
Helianthemum nummularium	0	68
Inula salicina	35	227
Linum tenuifolium	0	18
Lotus corniculatus	26	265
Onobrychis viciifolia	0	11
Peucedanum cervaria	36	167
Primula veris	5	105
Salvia nemorosa	4	43
Salvia pratensis	3	230
Salvia verticillata	25	168
Sanguisorba minor	14	241
Scabiosa ochroleuca	22	184
Securigera varia	43	301
Stachys recta	19	121
Tanacetum corymbosum	3	57
Teucrium chamaedrys	0	77

Table 2. List of traits involved in the study sorted in three groups according to what type of limitation they represent. The measure of colonization ability used as response variable is also presented. EIV = Ellenberg indicator value.

Trait group	Variable	Units	Minimum	Mean	Maximum	SD
Colonization ability (response)	Frequency in abandoned fields	%	0.0	30.3	95.7	30.7
Availability of seeds (A)	Frequency in grasslands	%	1.2	39.5	91.7	28.2
	Seed production	log(viable seeds/m^2)	1.594	2.842	3.731	0.475
	Length of flowering period	months	1	2.94	5	0.9
Seed dispersal traits (D)	Seed weight	log(mg)	-3.119	0.106	1.431	0.781
	Dispersal distance	log(m)	-0.263	0.257	1.184	0.263
	Exozoochory	%	0.0	12.7	80.0	20.2
	Endozoochory	%	21.3	70.5	100.0	23.0
Species habitat requirements (H)	Field germinability	%	0.0	6.5	24.1	6.1
	Field competitiveness	log(response ratio)	-0.427	0.070	0.825	0.297
	Niche width θ	-	5.4	7.8	9.0	0.9
	EIV Light	ordinal classes	6	7.2	9	0.7
	EIV Temperature	ordinal classes	5	6.1	8	0.6
	EIV Continentality	ordinal classes	2	4.3	6	1.0
	EIV Moisture	ordinal classes	2	3.6	7	1.1
	EIV Soil reaction	ordinal classes	7	8.2	9	0.8
	EIV Nutrients	ordinal classes	1	2.9	4	0.8

Table 3. Model selection of traits best explaining colonization ability. Species or PICs were used separately as items of evidence. Traits that best explained colonization ability were selected in forward regression based on F tests; F and *P* values shown are related to partial models in selection process. Selection was applied separately on each out of three groups of traits. Selected traits ($P < 0.05$) are in bold. EIV = Ellenberg indicator value.

	Species					Phylogenetically independent contrasts				
Trait group	Term	Df	Deviance	F	*P*	Term	Df	Deviance	F	*P*
Availability of seeds (A)	**Frequency in grasslands**	**1**	**11.460**	**78.29**	**< 0.001**	**Frequency in grasslands**	**1**	**1.648**	**92.03**	**< 0.001**
	Seed production	**1**	**0.671**	**4.59**	**0.041**	Seed production	1	0.019	1.08	0.306
	Length of flowering period	1	0.175	1.22	0.280	Length of flowering period	1	0.001	0.04	0.842
	Residual	28	4.265			Residual	29	0.517		
Seed dispersal traits (D)	Seed weight (log)	1	1.504	3.15	0.087	Dispersal distance (log)	1	0.216	3.29	0.080
	Dispersal distance (log)	1	1.010	2.11	0.158	Seed weight (log)	1	0.012	0.17	0.681
	Exozoochory	1	0.046	0.10	0.758	Exozoochory	1	0.046	0.67	0.420
	Endozoochory	1	0.007	0.01	0.907	Endozoochory	1	0.000	0.00	0.991
	Residual	27	14.004			Residual	27	1.913		
Species habitat requirements (H)	**Niche width**	**1**	**2.964**	**7.21**	**0.012**	**Niche width**	**1**	**0.459**	**7.98**	**0.008**
	EIV Moisture	1	1.523	4.02	0.054	**EIV Moisture**	**1**	**0.314**	**6.44**	**0.017**
	Field competitiveness	1	0.628	1.71	0.202	EIV Nutrients	1	0.073	1.53	0.226
	EIV Nutrients	1	0.451	1.27	0.269	EIV Soil Reaction	1	0.162	3.71	0.065
	EIV Soil Reaction	1	0.636	1.81	0.190	Field germinability	1	0.025	0.56	0.460
	Field germinability	1	0.528	1.49	0.233	Field competitiveness	1	0.041	0.92	0.347
	EIV Temperature	1	0.049	0.13	0.721	EIV Light	1	0.041	0.91	0.348
	EIV Continentality	1	0.053	0.14	0.712	EIV Temperature	1	0.023	0.50	0.485
	EIV Light	1	0.038	0.10	0.758	EIV Continentality	1	0.012	0.26	0.614
	Residual	22	9.701			Residual	22	1.036		

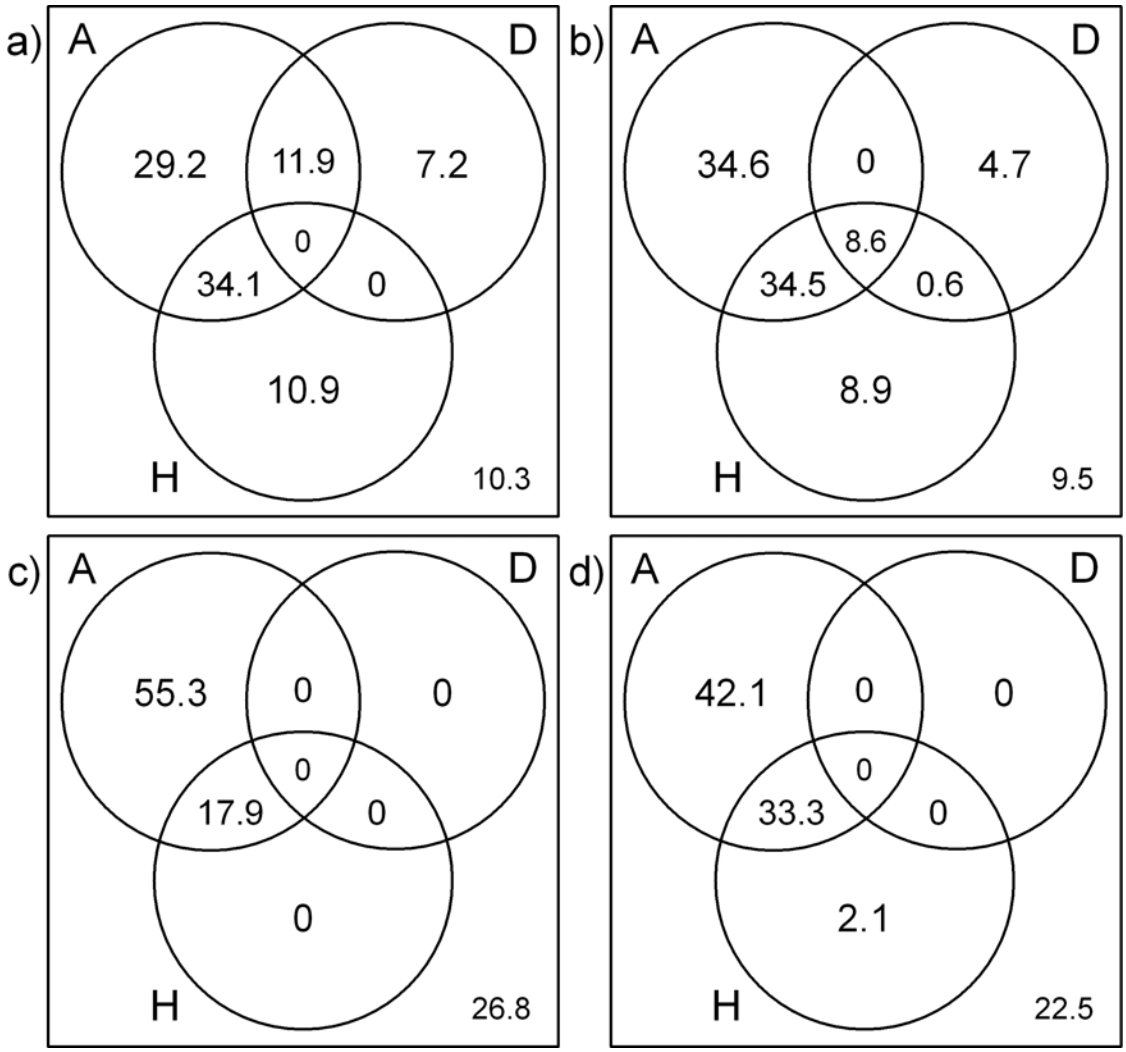


Fig. 1. Variance in colonization ability explained by availability of seeds (A), dispersal traits (D) and habitat requirements (H). Diagrams a, c are based on models using species and b, d on models using phylogenetically independent contrasts as items of evidence. Diagrams a, b are based on full models and c, d on best models originating from forward selection on individual trait groups (see Table 3). Percentage of variation attributed to each combination of variables is shown in respective segment; residual variability is shown in bottom right corner. Note that in diagrams a, b total variation exceeds 100%. This is due to the fact that some of the shared effects reported as being zero are actually negative. Negative variance values should be interpreted as null values, indicating correlation among the concerned explanatory variables and their opposite effect on response variable (Legendre & Legendre 1998).

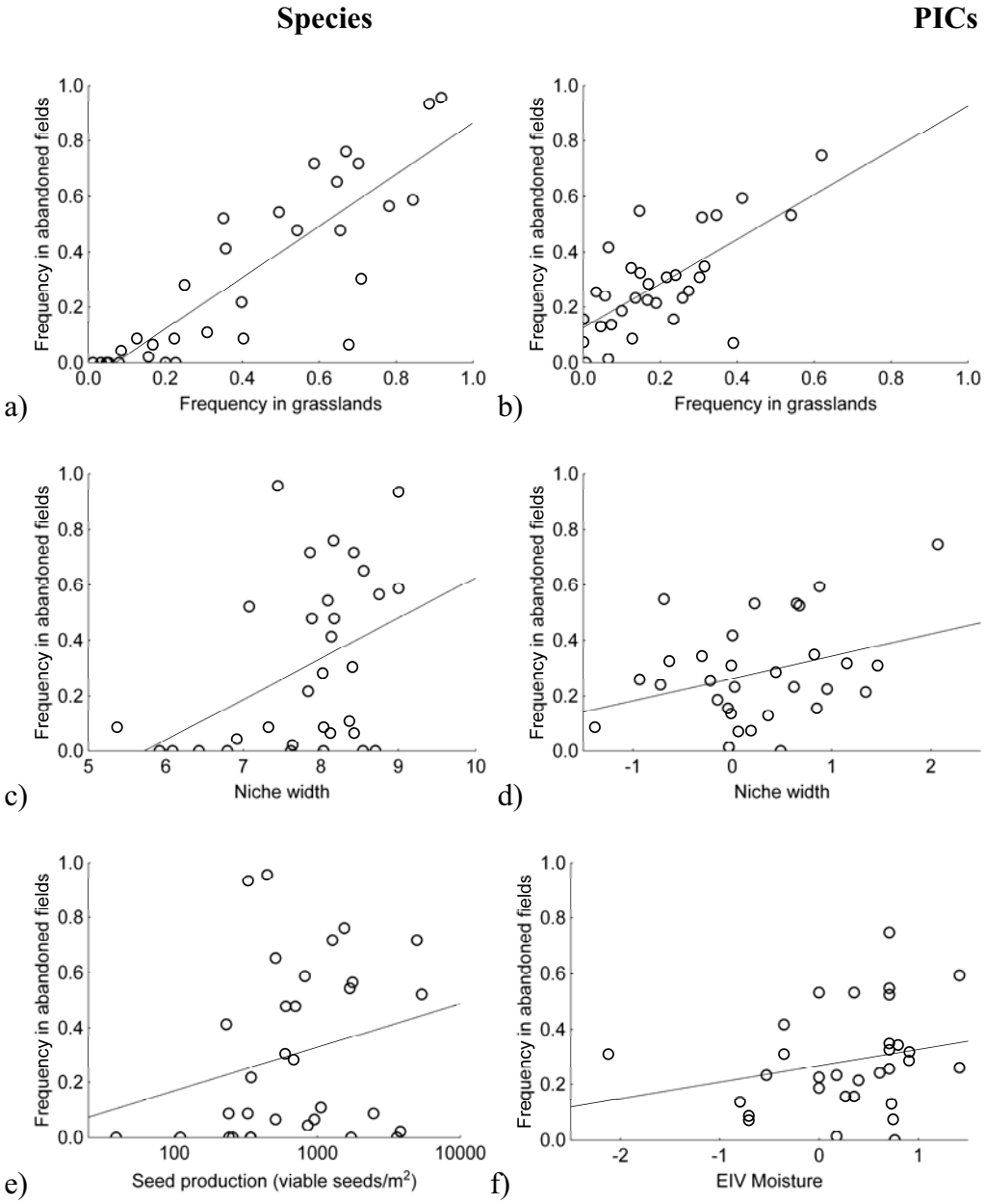


Fig. 2. Relationships between colonization ability (frequency in abandoned fields) and species traits which were selected in forward regressions as being significant. Circles in a,c,e represent species and circles in b,d,f represent PICs. Logarithmic scale on x axis is used in e.

References

Aerts, R. (1999) Interspecific competition in natural plant communities: mechanisms, trade-offs and plant-soil feedbacks. *Journal of Experimental Botany,* **50,** 29-37.

Bruun, H.H. (2000) Patterns of species richness in dry grassland patches in an agricultural landscape. *Ecography,* **23,** 641-650.

Cottrell, H.J. (1947) Tetrazolium Salt as a Seed Germination Indicator. *Nature,* **159,** 748-748.

Cousins, S.A.O. (2009) Landscape history and soil properties affect grassland decline and plant species richness in rural landscapes. *Biological Conservation,* **142,** 2752-2758.

Cousins, S.A.O., Ohlson, H. & Eriksson, O. (2007) Effects of historical and present fragmentation on plant species diversity in semi-natural grasslands in Swedish rural landscapes. *Landscape Ecology,* **22,** 723-730.

Diekmann, M. & Dupré, C. (1997) Acidification and eutrophication of deciduous forests in northwestern Germany demonstrated by indicator species analysis. *Journal of Vegetation Science,* **8,** 855-864.

Ehrlén, J. & Eriksson, O. (2000) Dispersal limitation and patch occupancy in forest herbs. *Ecology,* **81,** 1667-1674.

Ellenberg, H. (1988) *Vegetation ecology of Central Europe,* 4th edn. Cambridge University Press.

Ellenberg, H., Weber, H., Düll, R., Wirth, V., Werner, W. & Paulissen, D. (1992) *Zeigerwerte der Gefassplanzen Mitteleuropas,* 3nd edn. Erich Goltze KG, Gottingen.

Felsenstein, J. (1985) Phylogenies and the Comparative Method. *American Naturalist,* **125,** 1-15.

Freckleton, R.P. (2009) The seven deadly sins of comparative analysis. *Journal of Evolutionary Biology,* **22,** 1367-1375.

Freckleton, R.P., Harvey, P.H. & Pagel, M. (2002) Phylogenetic analysis and comparative data: A test and review of evidence. *American Naturalist,* **160,** 712-726.

Fridley, J.D., Vandermast, D.B., Kuppinger, D.M., Manthey, M. & Peet, R.K. (2007) Co-occurrence based assessment of habitat generalists and specialists: a new approach for the measurement of niche width. *Journal of Ecology,* **95,** 707-722.

Gleeson, S.K. & Tilman, D. (1994) Plant Allocation, Growth-Rate and Successional Status. *Functional Ecology,* **8,** 543-550.

Gomez-Aparicio, L., Gomez, J.M. & Zamora, R. (2007) Spatiotemporal patterns of seed dispersal in a wind-dispersed Mediterranean tree (Acer opalus subsp granatense): implications for regeneration. *Ecography,* **30,** 13-22.

Harvey, P.H. (1996) Phylogenies for ecologists. *Journal of Animal Ecology,* **65,** 255-263.

Harvey, P.H., Read, A.F. & Nee, S. (1995) Why Ecologists Need to Be Phylogenetically Challenged. *Journal of Ecology,* **83,** 535-536.

Hedges, L.V., Gurevitch, J. & Curtis, P.S. (1999) The meta-analysis of response ratios in experimental ecology. *Ecology,* **80,** 1150-1156.

Heywood, V.H. & Iriondo, J.M. (2003) Plant conservation: old problems, new perspectives. *Biological Conservation,* **113,** 321-335.

Chýlová, T. & Münzbergová, Z. (2008) Past land use co-determines the present distribution of dry grassland plant species. *Preslia,* **80,** 183-198.

Chytrý, M. & Rafajová, M. (2003) Czech National Phytosociological Database: basic statistics of the available vegetation-plot data. *Preslia,* **75,** 1-15.

Jongejans, E. & Schippers, P. (1999) Modeling seed dispersal by wind in herbaceous species. *Oikos,* **87,** 362-372.

Kleyer, M., Bekker, R.M., Knevel, I.C., Bakker, J.P., Thompson, K., Sonnenschein, M., Poschlod, P., van Groenendael, J.M., Klimeš, L., Klimešová, J., Klotz, S., Rusch, G.M., Hermy, M., Adriaens, D., Boedeltje, G., Bossuyt, B., Dannemann, A., Endels, P., Goetzenberger, L., Hodgson, J.G., Jackel, A.K., Kuehn, I., Kunzmann, D., Ozinga, W.A., Roemermann, C., Stadler, M., Schlegelmilch, J., Steendam, H.J., Tackenberg, O., Wilmann, B., Cornelissen, J.H.C., Eriksson, O., Garnier, E. & Peco, B. (2008) The LEDA Traitbase: a database of life-history traits of the Northwest European flora. *Journal of Ecology,* **96,** 1266-1274.

Knappová, J., Hemrová, L. & Münzbergová, Z. (2012) Colonization of central European abandoned fields by dry grassland species depends on the species richness of the source habitats: a new approach for measuring habitat isolation. *Landscape Ecology,* **27,** 97-108.

Kubát, K., Hrouda, L., Chrtek, J., Kaplan, Z., Kirschner, Z., Štěpánek , J. & Zázvorka, J. (2002) *Key to the Flora of the Czech Republic*. Academia, Praha.

Legendre, P. & Legendre, L. (1998) *Numerical ecology,* 2nd English edn. Elsevier, Amsterdam.

Manthey, M. & Fridley, J.D. (2009) Beta diversity metrics and the estimation of niche width via species co-occurrence data: reply to Zeleny. *Journal of Ecology,* **97,** 18-22.
Münzbergová, Z. (2004) Effect of spatial scale on factors limiting species distributions in dry grassland fragments. *Journal of Ecology,* **92,** 854-867.
Münzbergová, Z. (2005) Determinants of species rarity: Population growth rates of species sharing the same habitat. *American Journal of Botany,* **92,** 1987-1994.
Münzbergová, Z. & Herben, T. (2005) Seed, dispersal, microsite, habitat and recruitment limitation: identification of terms and concepts in studies of limitations. *Oecologia,* **145,** 1-8.
Murray, B.R., Thrall, P.H., Gill, A.M. & Nicotra, A.B. (2002) How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecology,* **27,** 291-310.
Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N. & Pearse, W. (2012) caper: Comparative Analyses of Phylogenetics and Evolution in R.
Öster, M., Ask, K., Cousins, S.A.O. & Eriksson, O. (2009) Dispersal and establishment limitation reduces the potential for successful restoration of semi-natural grassland communities on former arable fields. *Journal of Applied Ecology,* **46,** 1266-1274.
Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature,* **401,** 877-884.
Pagel, M.D. (1992) A Method for the Analysis of Comparative Data. *Journal of Theoretical Biology,* **156,** 431-442.
Pocock, M.J.O., Hartley, S., Telfer, M.G., Preston, C.D. & Kunin, W.E. (2006) Ecological correlates of range structure in rare and scarce British plants. *Journal of Ecology,* **94,** 581-596.
Poschlod, P. & Bonn, S. (1998) Changing dispersal processes in the central European landscape since the last ice age: an explanation for the actual decrease of plant species richness in different habitats? *Acta Botanica Neerlandica,* **47,** 27-44.
R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Revell, L.J. (2010) Phylogenetic signal and linear regression on species data. *Methods in Ecology and Evolution,* **1,** 319-329.
Ruprecht, E. (2006) Successfully recovered grassland: A promising example from Romanian old-fields. *Restoration Ecology,* **14,** 473-480.
Soons, M.B. & Heil, G.W. (2002) Reduced colonization capacity in fragmented populations of wind-dispersed grassland forbs. *Journal of Ecology,* **90,** 1033-1043.
Terborgh, J., Alvarez-Loayza, P., Dexter, K., Cornejo, F. & Carrasco, C. (2011) Decomposing dispersal limitation: limits on fecundity or seed distribution? *Journal of Ecology,* **99,** 935-944.
Tremlová, K. & Münzbergová, Z. (2007) Importance of species traits for species distribution in fragmented landscapes. *Ecology,* **88,** 965-977.
Turnbull, L.A., Crawley, M.J. & Rees, M. (2000) Are plant populations seed-limited? A review of seed sowing experiments. *Oikos,* **88,** 225-238.
Uriarte, M., Bruna, E.M., Rubim, P., Anciaes, M. & Jonckheere, I. (2010) Effects of forest fragmentation on the seedling recruitment of a tropical herb: assessing seed vs. safe-site limitation. *Ecology,* **91,** 1317-1328.
Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. & Garnier, E. (2007) Let the concept of trait be functional! *Oikos,* **116,** 882-892.
Vítová, A. & Lepš, J. (2011) Experimental assessment of dispersal and habitat limitation in an oligotrophic wet meadow. *Plant Ecology,* **212,** 1231-1242.
Volkov, I., Banavar, J.R., Hubbell, S.P. & Maritan, A. (2003) Neutral theory and relative species abundance in ecology. *Nature,* **424,** 1035-1037.
Web 1. Angiosperm, Phylogeny Website. http://www.mobot.org/MOBOT/research/APweb/ 24. 5. 2012
Webb, C.O. & Donoghue, M.J. (2005) Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes,* **5,** 181-183.
Zelený, D. (2009) Co-occurrence based assessment of species habitat specialization is affected by the size of species pool: reply to Fridley et al. (2007). *Journal of Ecology,* **97,** 10-17.

SUPPORTING INFORMATION

Appendix S1 *Testing for degree of phylogenetic dependence.*

Figure S1 *Phylogenetic tree of target species.*

Table S1 *Correlation of traits based on cross-species comparison and PICs.*

Appendix S1. Testing for degree of phylogenetic dependence

For all variables (both dependent and independent) under study we calculated Pagel's λ (Freckleton *et al.*, 2002; Pagel, 1999) using function `fitContinuous` in R package `'geiger'` (Harmon *et al.*, 2009). A value of $\lambda = 0$ indicates evolution of traits that is independent of phylogeny, while a value of $\lambda = 1$ indicates that traits are evolving according to Brownian motion on the given phylogeny. Intermediate values of λ indicate that traits have evolved according to a process in which the effect of phylogeny is weaker than in the Brownian model (Pagel, 1999). The estimate of λ was tested in order to determine whether data exhibit significant phylogenetic dependence or not with the log-likelihood ratio test with χ^2 distribution of test statistic (Freckleton *et al.*, 2002).

Species occurrence in abandoned fields and eight out of 15 investigated traits showed nearly random distribution across phylogeny ($\lambda < 0.001$; see table below). Occurrence in abandoned fields and twelve traits also exhibited degree of phylogenetic dependence being significantly different from Brownian model (λ significantly lower than 1; Table). Only one trait (Ellenberg indicator value for moisture) was significantly different from random distribution across phylogeny (λ significantly higher than 0; see table below).

Species traits involved in the study sorted according to what type of limitation they represent. A value of $\lambda = 1$ indicates that trait values are distributed in a manner consistent with the Brownian model of trait evolution, whereas a value of $\lambda = 0$ suggests that traits co-vary independently of phylogeny. Intermediate values of λ indicate varying degrees of phylogenetic dependence in the data. The maximum likelihood estimate of λ (λ = ML) is given together with its associated log likelihood (ln lik). Also shown are the log-likelihood values for the model, with λ set to either 0 or 1. Values significantly different from the test value ($P < 0.05$ determined from a log-likelihood ratio test) are indicated in bold.

	λ = ML	ln lik (λ =ML)	ln lik (λ =0)	ln lik (λ =1)
Response				
Frequency in abandoned fields	< 0.001	-7.085	-7.085	**-15.244**
Availability of seeds				
Frequency in grasslands	< 0.001	-5.180	-5.180	**-13.098**
Seed production	< 0.001	-22.123	-22.123	**-25.229**
Length of flowering period	< 0.001	-44.508	-44.508	**-47.762**
Dispersal traits				
Seed weight	1.000	-39.544	**-43.805**	-39.544
Dispersal distance	0.914	0.056	**-4.556**	-0.344
Exozoochory	< 0.001	-160.041	-160.041	**-317.063**
Endozoochory	0.696	2.250	0.864	1.841
Habitat requirements				
Field germinability	< 0.001	41.159	41.159	**38.865**
Field competitiveness	0.423	-4.145	-5.185	**-6.187**
Niche width	< 0.001	-44.835	-44.835	**-50.148**
Light	< 0.001	-36.636	-36.635	**-41.770**
Temperature	1.000	-32.264	**-34.592**	-32.264
Continentality	< 0.001	-46.689	-46.689	**-52.583**
Moisture	1.000	-47.086	**-53.062**	-47.086
Soil Reaction	< 0.001	-38.423	-38.423	**-44.068**
Nutrients	0.099	-38.578	-38.644	**-41.531**

References

Freckleton, R.P., Harvey, P.H., & Pagel, M. (2002) Phylogenetic analysis and comparative data: A test and review of evidence. *American Naturalist*, **160**, 712-726.

Harmon, L., Weir, J., Brock, C., Glor, R., Challenger, W., & Hunt, G. (2009) geiger: Analysis of evolutionary diversification.

Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature*, **401**, 877-884.

Figure S1: Phylogenetic tree of target species.

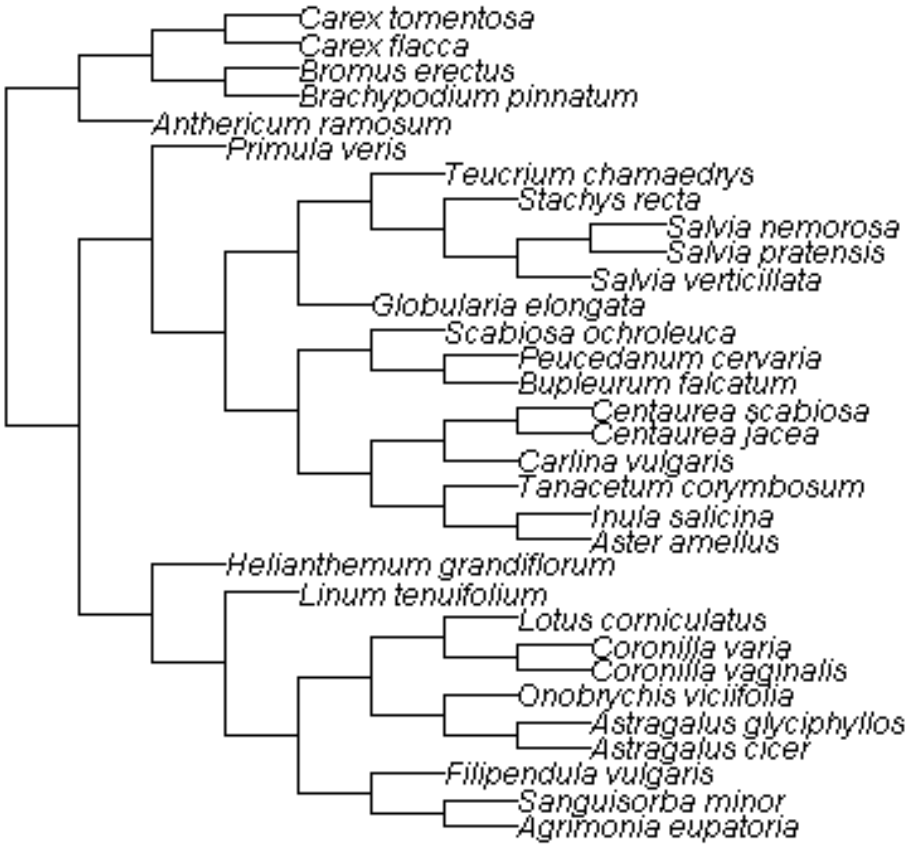


Table S1. Correlation of traits based on cross-species comparison (1) and PICs (2). Sigificant correlations ($P <$ 0.05) are in bold.

(1)

Trait group		Variable	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
Colonization ability	(1)	Frequency in fields																
Availability of seeds	(2)	Frequency in grasslands	**0.86**															
	(3)	Seed production	0.25	0.14														
	(4)	Length of flowering period	0.06	0.02	**-0.42**													
Seed dispersal traits	(5)	Seed weight (log)	0.30	0.25	-0.12	0.29												
	(6)	Dispersal distance (log)	0.22	0.16	0.34	-0.06	0.06											
	(7)	Exozoochory	0.19	0.07	0.06	-0.06	0.08	0.43										
	(8)	Endozoochory	0.03	0.06	0.28	-0.22	0.20	-0.11	-0.22									
Species habitat requirements	(9)	Field germinability	0.27	0.28	0.12	0.30	0.15	0.25	0.08	-0.03								
	(10)	Field competitiveness	0.12	-0.11	0.13	0.20	0.19	-0.26	-0.16	0.21	-0.22							
	(11)	Niche width	**0.42**	**0.50**	0.26	0.09	-0.04	0.15	-0.01	-0.24	**0.36**	0.03						
	(12)	EIV Light	-0.24	-0.21	-0.06	0.07	-0.14	0.12	0.00	-0.03	-0.03	**-0.36**	-0.35					
	(13)	EIV Temperature	-0.18	-0.25	-0.08	-0.11	-0.15	-0.10	-0.11	0.02	-0.17	-0.04	**-0.36**	0.33				
	(14)	EIV Continentality	0.05	-0.05	-0.07	-0.01	0.08	0.01	0.07	0.13	**-0.36**	0.09	0.02	0.04	0.33			
	(15)	EIV Moisture	0.28	0.25	0.12	**-0.40**	-0.10	**0.38**	0.28	0.33	-0.01	-0.20	-0.06	-0.25	-0.14	-0.03		
	(16)	EIV Soil Reaction	-0.03	-0.17	-0.34	0.06	-0.06	-0.19	0.07	-0.02	-0.34	-0.02	**-0.39**	0.35	0.32	0.31	0.01	
	(17)	EIV Nutrients	0.26	**0.37**	0.25	-0.18	0.03	**0.36**	0.28	0.03	0.27	**-0.40**	0.15	-0.18	-0.12	-0.20	**0.44**	-0.34

(2)

Trait group		Variable	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
Colonization ability	(1)	Frequency in fields																
Availability of seeds	(2)	Frequency in grasslands	**0.68**															
	(3)	Seed production	0.13	0.01														
	(4)	Length of flowering period	-0.01	0.01	**-0.37**													
Seed dispersal traits	(5)	Seed weight (log)	0.26	0.24	-0.14	0.27												
	(6)	Dispersal distance (log)	0.29	0.15	0.30	0.06	0.22											
	(7)	Exozoochory	0.29	-0.01	-0.01	-0.05	0.19	0.19										
	(8)	Endozoochory	-0.22	-0.12	**0.41**	-0.28	0.14	-0.05	-0.15									
Species habitat requirements	(9)	Field germinability	0.08	0.05	0.16	0.28	0.12	0.25	0.10	-0.14								
	(10)	Field competitiveness	-0.04	-0.30	0.10	0.08	0.06	-0.10	-0.01	0.04	-0.27							
	(11)	Niche width	0.33	**0.46**	0.17	0.07	-0.05	0.08	-0.10	-0.27	0.30	0.02						
	(12)	EIV Light	-0.07	-0.03	-0.02	0.18	0.05	0.16	-0.06	0.22	0.11	**-0.38**	-0.34					
	(13)	EIV Temperature	-0.04	-0.07	-0.09	-0.21	-0.13	-0.02	-0.14	0.26	0.01	-0.19	-0.29	0.21				
	(14)	EIV Continentality	0.20	0.15	-0.15	-0.11	0.11	-0.18	-0.04	0.19	**-0.36**	-0.02	0.12	-0.02	0.24			
	(15)	EIV Moisture	0.24	0.07	0.14	**-0.46**	-0.21	0.30	0.30	0.12	-0.22	-0.18	-0.03	-0.20	0.11	0.03		
	(16)	EIV Soil Reaction	**0.37**	0.15	-0.31	0.04	0.01	-0.20	0.13	0.08	-0.21	-0.04	-0.29	0.27	0.18	0.30	0.19	
	(17)	EIV Nutrients	0.05	0.08	0.20	-0.08	0.06	0.16	0.26	-0.03	0.12	-0.31	-0.03	-0.18	0.13	-0.12	0.22	-0.27

Low seed pressure restricts dry grassland specialists to edges of abandoned fields

Jana Knappová & Zuzana Münzbergová
manuscript

Low seed pressure restricts dry grassland specialists to edges of abandoned fields

Jana Knappová, Zuzana Münzbergová

Department of Botany/Ecology, Faculty of Science, Charles University in Prague, Benátská 2, 128 01, Prague, Czech Republic
and
Institute of Botany, Academy of Sciences of the Czech Republic, 252 43, Průhonice, Czech Republic

Abstract

Abandoned fields are perceived as alternative habitats for species from declining grasslands, but former cultivation might considerably alter habitat conditions making them unsuitable for specialist grassland species. In present study, we asked whether abandoned fields in northern Czech Republic represent suitable habitat for dry grassland species and what limits their successful colonization.
We compared occurrence of 66 dry grasslands species in edges and interiors of abandoned fields. More species occurred in the edges than in the interiors. Source quality of neighbouring habitat had positive effect on species richness in edges and it was insignificant in interiors. Although edges were found to be slightly warmer and drier than interiors, they were not preferably colonized by species with higher temperature or low moisture demands. It is possible that rather than favouring species according to their habitat requirements, warmer and drier conditions make resident vegetation less dense providing more microsites for establishment. Therefore, compare to interiors edges could be colonized also by species with low seed pressure, which are unable to reach scarce microsites in denser field interiors. Indeed, species that occur almost evenly in edges and interiors are species frequent in dry grasslands within the study area whereas species restricted to field edges occur sporadically in dry grasslands.
Species with high light demands and low nutrient, moisture and soil reaction demands and species less frequent in dry grasslands were more restricted to field edges neighbouring with habitat of high quality. All these traits characterize grassland specialists, which are unlikely to occur in low quality source habitats.
Abandoned fields in the study region could be successfully colonized by species that are also common in dry grasslands. For these species, abandoned fields represent suitable habitat and can potentially enhance their landscape dynamics. However, rare dry grassland species occur only sporadically in these fields and mainly in edges only. Our results indirectly indicate that low microsite availability together with low seed pressure is most limiting for these species. They are therefore much more restricted to dry grasslands and their conservation mostly relies on proper management of their current habitats.

Keywords: dispersal traits, distribution, fallow land, habitat requirements, species richness, succession

Introduction

The extent of species-rich semi-natural grasslands has been drastically declining throughout Europe over the past century, and the remaining grasslands are fragmented and scattered within the landscape (Emanuelsson, 2008). Cessation of former management at some sites and management intensification at other sites has led to the deterioration of habitat conditions and decreased species richness (Eriksson *et al.*, 2002; Luoto *et al.*, 2003; Cousins, 2009). In recent decades, socio-economic changes and new technologies employed in agriculture have brought about the abandonment of arable fields that were no longer profitable to maintain (Cramer *et al.*, 2008). Abandoned fields represent open, treeless habitats, and they are hence perceived as alternative habitats for species from declining grasslands (Walker *et al.*, 2004; Öster *et al.*, 2009a,b; Knappová *et al.*, 2012).

Legacies of former cultivation might considerably alter habitat conditions on former fields making them unsuitable for specialist grassland species (Janssens *et al.*, 1998; Cramer *et al.*, 2008). However, when seed input from source populations is high enough, plants could (repeatedly) colonize habitats, which are actually unsuitable for their long term persistence (Eriksson, 1996). At the same time, suitable habitats might stay unoccupied due to lack of available seeds (Münzbergová & Herben, 2005) making it difficult to join environmental characteristics with species occurrences (Ozinga *et al.*, 2005). It is thus clear that identification of suitable habitats is constrained by existence of both suitable unoccupied and unsuitable occupied habitats. Seed addition experiments might be helpful in order to asses habitat suitability (Turnbull *et al.*, 2004; Öster *et al.*, 2009b), but they are hardly applicable routinely on a large scale due to their high time and work demands.

In present study, we investigate the ongoing process of colonization of recently abandoned arable fields by species from current grasslands in northern Bohemia (Czech Republic). A previous work in the same region demonstrated some portions of the current area of dry grasslands were arable fields in the 1950's or even in 1980's (Chýlová & Münzbergová, 2008), suggesting that grassland species have been able to spread into novel habitats. Furthermore, many grassland species also already occur in recently abandoned fields (Knappová *et al.*, 2012). We therefore assume that abandoned fields in this area represent potentially suitable habitats for grassland species.

Fields in our study have been abandoned approximately 20 years ago. We expect that this time is long enough for species to reach not only the closest field edge but (given that abandoned field represents suitable habitat) also to spread further into field interior. In case of unconstrained colonization, edges and interiors should have the same species richness and species composition (with the condition that edges and interiors exhibit similar environmental characteristics). Subsequently, possible differences in certain traits of species prevailing in edges compared to species in the field interior could provide important insights in factors limiting colonization. Differences in dispersal traits would suggest that for species with poor dispersal twenty years is not enough even to overcome distance of few meters from edge to interior. Alternatively, abandoned fields could be rather unsuitable (sink) habitat for grassland species and their populations here are maintained by seed supply from nearby sources. Differences in habitat requirements would suggest either difference in site conditions or again

unsuitability of abandoned fields for species occurring predominantly in edges. Differences in seed availability (i.e. in species frequency in source grasslands) would imply importance of sufficient seed supply for successful colonization.

To find out factors that most influence colonization of abandoned fields, we aim to answer following questions:

1) How does species richness and species composition of grassland plants differ between edges and interiors of abandoned fields?

2) How does species richness of grassland plants in field interiors depend on richness in field edges?

3) Can differences in species colonization ability (expressed as species affinity to field edges) be attributed to differences in dispersal traits, habitat requirements or seed availability?

Small grassland herbs are only rarely adapted to long distance wind dispersal (Eriksson & Jakobsson, 1998) and their travelling distances might be limited to few meters (Öster *et al.*, 2009b). We already demonstrated that distant seed sources (hundreds of meters) are of minor importance for species richness in studied abandoned fields (Knappová *et al.*, 2012). Similar conclusion about insignificance of long-distance dispersal in grassland plants was made by Maurer *et al.* (2003). In this light, source habitats in the closest neighbourhood of abandoned fields are likely to be the most important for their colonization. Seed input into target fields would therefore to large extent depend on quality of these seed sources. Nonetheless, some species might be more restricted to high quality source habitats than others. We therefore ask:

4) How does species richness and species composition of grassland plants differ between field edges according to quality of neighbouring source habitat?

5) Can differences in species affinity to high quality of neighbouring habitat be attributed to differences in dispersal traits, habitat requirements or seed availability?

Methods

Study region and target species

Study was performed in the northern part of the Czech Republic in an area delimited by towns Litoměřice, Ústěk and Roudnice nad Labem. Natural vegetation in the region is represented by remnants of oak-hornbeam and thermophilous oak forests (alliance *Carpinion* and *Quercion petrae*; Ellenberg, 1988). Semi-natural calcareous dry grasslands (alliance *Bromion erecti*; Ellenberg, 1988) occur in small fragments and they host a vast small-scale diversity of vascular plants (Münzbergová, 2004; Chýlová & Münzbergová, 2008), including a number of threatened species. At present, most of the grasslands in the region are not managed, and occasionally, some of them are completely destroyed by human activities (e.g., ploughing or development of solar power plants). Fields abandoned in the last 20 years are already overgrown with grasses and ruderal herbaceous vegetation, e.g., *Arrhenatherum elatius, Dactylis glomerata, Cirsium arvense* and *Melilotus spp*. In total, 48 abandoned fields were included in the dataset, ranging in size from 814 m^2 to 79 208 m^2 (median 6 553 m^2).

Based on studies performed within the same region (Münzbergová, 2004; Tremlová & Münzbergová, 2007; Chýlová & Münzbergová, 2008), we selected 87 target species as species restricted to dry grassland fragments (see also

Knappová *et al.*, 2012). Only 66 species were found in surveyed abandoned fields and hereafter, only these are referred to as target species (Table 1).

Data collection

In all of the abandoned fields, the occurrence of each of the 66 target species was recorded during 2008 or 2009. The occurrence was recorded separately for interior of the field and for edge zone 5 m wide. The edge zone was further divided into segments according to the type of adjacent habitat. Individual edges were divided into 1-5 segments (median 3).

For each habitat type, source quality was assessed as a measure of the potential of individual habitat to serve as source of propagules of target species. Dry grasslands, extensively managed gardens or orchards represented habitats of high quality and abandoned fields, intensively managed gardens or meadows, fields, forests, shrubs and built-up areas represented habitats of low quality.

All target abandoned fields and their divisions into interior segments and sectional edge zone were localized in ortofotomap. All segments were then digitized and their area was calculated using ArcGIS 9.2 (ESRI, 2006). Edge zones ranged in size from 587 to 11 130 m^2 (median 2 298 m^2). Interiors ranged in size from 227 to 68 077 m^2 (median 4 908 m^2). Edge segments ranged in size from 48 to 5 405 m^2 (median 665 m^2).

To evaluate possible differences in environmental conditions among edges and interiors, we used data on Ellenberg indicator values (Ellenberg *et al.*, 1992) for light, temperature, continentality, moisture, soil reaction and nutrients. The values are simple ordinal classes indicating species optima of realized ecological niche along a gradient. The indicator values were developed mainly on the basis of field experience and reflect the ecological behaviour of species (Diekmann & Dupré, 1997).

Data analysis

First, to evaluate general diversity pattern, we used generalised linear mixed-effect model (glmm; function glmer in package lme4; Bates *et al.*, 2011) with Poisson distribution of errors. Number of target species in each segment was used as dependant variable, spatial position (edge/interior) as explanatory variable, and field code as random term. In this analysis, whole edge zone was used without further division. We found significant relationship among log-transformed area and number of species in edges or interiors (data not shown). Therefore, to account for size differences among sites, area (log-transformed) was used as covariable.

We used glmm with Poisson distribution also to evaluate the effect of source quality on number of target species in the splitted edge segments. Source quality was used as fixed effect and field code as random term. The effect of source quality on number of target species in edges was tested also on more rough scale, with whole edge zones. In this case we used glm with quasi-Poisson distribution, due to overdispersion in data. Number of species was used as dependent variable, maximum source quality in field surroundings as independent variable and area (log-transformed) was used as covariable. The same analysis was performed for field interiors. The relationship between species richness of edges and interiors was tested with linear regression. Species richness was expressed as number of species per area (log-transformed).

To evaluate possible differences in habitat conditions among edges and interiors, we calculated Ellenberg indicator values for each site as mean value of all species occurring at this site. Mean values of each out of six indicators were then compared with glmm with Gaussian distribution, spatial position (edge/interior) was used as fixed effect and field code as random term.

To test whether there is any difference in species composition between edges and interiors of the fields, we performed canonical correspondence analysis (CCA) with downweighting of rare species (Lepš & Šmilauer, 2003). Spatial position (edge/interior) was used as environmental (independent) variable. In this analysis, whole edge zone was used without further division. Significance was tested using Monte-Carlo permutation tests within blocks defined by field code as covariable.

To test for the effect of quality of seed source on species composition in edges, further CCA was performed with source quality used as environmental variable (maximum source quality in field surroundings was used for the whole edge). Finally, to refine resolution, similar analysis was performed with splitted edge segments, to evaluate the effect of shrub barrier and source quality on species composition. In this case, edge permeability, source quality and their interaction were used as environmental variables. Significance was tested using Monte-Carlo permutation test with unrestricted permutations.

In addition, we test for differences in species traits that could explain species affinity to field edges. We used sores on first (canonical) axis from CCA where edge and interior were used as explanatory variables. These numbers express to what extent species occurrence was limited to field edges. Negative scores indicate species were found predominantly in edges whereas species with scores around and slightly above zero were found approximately equally often in edges and interiors. CCA scores were than correlated with trait values of individual species (Appendix 1). To test for differences among species more or less bounded to field edges neighbouring with high quality habitat, we used sores on first (cannonical) axis from CCA where source quality was used as explanatory variable. Again, CCA scores were than correlated with trait values of individual species.

Only species occurring in more than one abandoned field were included in multivariate analysis (hence 8 species were excluded). These species were also excluded from trait analysis. CCAs were performed in Canoco for Windows 4.5 (Ter Braak & Šmilauer, 2002). All univariate analyses were performed in R 2.14.1 (R Development Core Team, 2011).

Results

Out of 66 target species, 11 species occurred exclusively in the edges (Table 1). Significantly more species occurred in the edges than in the interiors ($z = 8.121$, $P < 0.001$; Figure 1), which was also expressed in slope of relationship between species richness in edges and species richness in interiors (Figure 2). This relationship was also highly significant ($z = 8.376$, $P < 0.001$).

Source quality of neighbouring habitat did not affected significantly species richness of splitted edge segments ($z = 1.220$, $P = 0.223$). At more rough scale of whole edges, however, source quality had significantly positive effect on species richness ($F_{1,45} = 14.936$, $P < 0.001$; Figure 3) suggesting that source

habitats influence not only the closest edges. Effect of source quality was insignificant in interiors ($F_{1,45} = 2.759$, $P = 0.104$; Figure 3).
When mean Ellenberg indicator values were compared edges were found to be significantly warmer and drier than interiors (Appendix 2).

Species composition differed significantly among edges and interiors ($P = 0.001$, 999 permutations) and spatial position within field explained 4.2% of variation in species data (Figure 4). At coarser scale of whole edges, there was significant effect of source quality ($P = 0.001$; Figure 5) and it explained 4.3% of variation in species data. Source quality at finer scale of splitted edge segments had only marginal effect ($P = 0.053$) and explained 1.3% of variation in species data.

Neither trait related to dispersal or to habitat requirements was correlated with CCA scores expressing species affinity to field edges (Appendix 3). Significant positive correlations were found only with species frequency in dry grasslands (Appendix 3, Figure 6). Four out of six Ellenberg indicator values representing habitat requirements were significantly correlated with CCA scores expressing species affinity to high quality source habitat (Appendix 3). Species with high light demands and low nutrient, moisture and soil reaction demands were more restricted to field edges neighbouring with habitat of high quality (Figure 7). Significant positive correlations were found also with species frequency in dry grasslands.

Discussion

Abandoned fields provide interesting study system for testing ecological theory but also an important challenge for the practice of ecological restoration (Cramer *et al.*, 2008). At certain circumstances, field succession might lead to grasslands of high conservation value with no or minimum human intervention (Jongepierová *et al.*, 2004; Ruprecht, 2006). In most cases, however, colonization of abandoned fields by desirable (e.g. grassland) species is strongly limited by both habitat conditions and seed availability (Buisson *et al.*, 2006; Foster *et al.*, 2007; Öster *et al.*, 2009b; Knappová *et al.*, 2012).

Fields surveyed in our study host considerable amount of dry grassland species. Field interiors, however, were undersaturated with target dry grassland species compared to field edges (Figure 1,2). It could be, that time since abandonment (approximately 20 years) was not long enough for complete colonization of field interiors as we had originally anticipated. However, some species occur almost evenly in interiors as well as in edges, whereas others were found mostly in edges (Table 1, Figure 4). This difference in species composition among edges and interiors indicates that inter-specific variation in colonization ability rather than generally short time span is responsible for incomplete colonization of field interiors.

We expected that difference among species is given either by their ability to disperse (poor dispersers would occur predominantly in edges) or by their habitat requirements (abandoned fields are rather unsuitable habitat for species occurring mainly in the edges and their populations here are maintained by seed supply from nearby sources). However, neither traits related to dispersal nor habitat requirements explained differences among species in their affinity to field edges (Appendix 3). It is especially striking that although edges were found to be slightly warmer and drier than interiors (Appendix 2), they were not preferably

colonized by species with higher temperature or low moisture demands. It is possible that rather than favouring species according to their habitat requirements, warmer and drier conditions make resident vegetation less dense providing more microsites for establishment. Therefore, compare to interiors edges could be colonized also by species with low seed pressure, which are unable to reach scarce microsites in denser field interiors. Indeed, species that occur almost evenly in edges and interiors are species frequent in dry grasslands within the study area whereas species restricted to field edges occur sporadically in dry grasslands.

We also can not exclude another possibility, that despite a wide range of tested traits we could miss some other important traits which are responsible for species rarity through influencing colonization ability (Murray *et al.*, 2002; Maurer *et al.*, 2003). Nevertheless, similar independence of colonization ability regarding diverse traits was reported in case of grazed abandoned fields in Scandinavia (Öster *et al.*, 2009a). Due to relatively poor competitive ability of grassland species compare to ruderal vegetation of the fields, successful colonization should mainly depends on timely occupation of gaps or open spaces (Bartha *et al.*, 2003). Obviously, the more seeds are available, the higher is their probability of capturing suitable microsites. Unfortunately, we do not have data on seed production for involved species and frequency in dry grasslands therefore remains the only proxy of seed pressure.

Colonization of abandoned fields is often constrained by availability of seeds which clearly depends on quality and proximity of source habitats (Cramer *et al.*, 2008). Several authors proposed methods how to evaluate habitat quality in terms of seed supply (Ruprecht, 2006; Knappová *et al.*, 2012). We distinguished habitats only into two groups – high and low source quality. Despite this simplified method, we found pronounced effect of source quality on species richness and species composition of grassland plants in field edges. Nonetheless, the effect was significant only on more rough scale of whole edges. This implies that seed dispersal from particular source habitat might not be directed only towards the nearest edge segment but rather source habitat of high quality could influence larger part of the field edge. Alternatively, our failure to find the effect of source quality on finer scale of edge segments might be due to relatively low numbers of these segments within individual fields (1-5, median 3).

The effect of source quality on species richness, however, diminished with distance from edge into interiors (Figure 3). This suggests that large fraction of species colonizing preferably field edges also depends on neighbourhood of high quality habitat whereas species that often colonize even interiors mainly occur independently on source habitat quality. Species with high light demands and low nutrient, moisture and soil reaction demands were more restricted to field edges neighbouring with habitat of high quality (Figure 7). All these traits characterize grassland specialists, which are unlikely to occur in low quality source habitats. Although these species seem to be dispersal limited we did not find any significant difference in dispersal traits. Possible explanation is that lack of seeds resulting in colonization failure is not given primarily by dispersal *per se* (represented by dispersal traits), but to seed availability in landscape context (i.e., number of source populations or seed production in these populations) resulting in higher seed pressure. This explanation is in agreement with above discussed findings that species affinity to edges is related to traits describing rarity/commonness and also with our further research on this topic (Knappová et al. *submitted*).

Further usage and fate of abandoned fields depends to large extent on our understanding of mechanisms that underlie their colonization. We proved that abandoned fields in the study region could be successfully colonized by species that are also common in dry grasslands. For these species, abandoned fields represent suitable habitat and can enhance their landscape dynamics. However, rare dry grassland species occur only sporadically in these fields and mainly in edges only. Our results indirectly indicate that low microsite availability together with low seed pressure is most limiting for these species. They are therefore much more restricted to dry grasslands and their conservation mostly relies on proper management of their current habitats.

Acknowledgments

We thank to Z. Červenková, L. Hemrová and M. Knapp for their assistance during the field survey. This study was supported by the Grant Agency of Czech Republic (Grants no. P505/10/0593 and P504/10/0456), and partly by the Academy of Sciences of the Czech Republic (Grant no. RVO 67985939).

Table 1. List of 66 target dry grassland species occurring in 48 surveyed abandoned fields and number of their occurrences in edge and interior. Numbers in brackets denote in how many fields individual species occurred solely in edge or interior. Species in bold were found solely in edges. Nomenclature follows Tutin *et al.* (1964-1980).

Species	Number of occurrences		Species	Number of occurrences	
	Edges	Interiors		Edges	Interiors
Agrimonia eupatoria	46 (8)	39 (1)	*Knautia arvensis*	35 (17)	19 (1)
Anthyllis vulneraria	1 (0)	1 (0)	*Koeleria macrantha*	4 (3)	1 (0)
Artemisia campestris	2 (0)	2 (0)	***Laserpitium latifolium***	1 (1)	0 (0)
Asperula cynanchica	3 (2)	1 (0)	*Leontodon hispidus*	6 (4)	2 (0)
Aster amellus	4 (2)	2 (0)	*Linum catharticum*	18 (9)	12 (3)
Astragalus cicer	21 (8)	15 (2)	*Linum flavum*	1 (0)	1 (0)
Astragalus glycyphyllos	32 (3)	32 (3)	*Lotus corniculatus*	28 (13)	17 (2)
Brachypodium pinnatum	28 (12)	16 (0)	*Medicago falcata*	2 (2)	2 (2)
Briza media	4 (3)	1 (0)	*Melampyrum arvense*	4 (3)	1 (0)
Bromus erectus	10 (5)	7 (2)	*Melampyrum nemorosum*	1 (0)	1 (0)
Bupleurum falcatum	30 (15)	20 (5)	***Onobrychis viciifolia***	6 (6)	0 (0)
Carex flacca	2 (2)	0 (0)	*Ononis spinosa*	2 (1)	1 (0)
Carex humilis	1 (1)	0 (0)	*Origanum vulgare*	34 (6)	30 (2)
Carex tomentosa	1 (1)	1 (1)	*Peucedanum cervaria*	4 (3)	1 (0)
Carlina vulgaris	16 (5)	11 (0)	*Pimpinella saxifraga*	6 (2)	5 (1)
Centaurea jacea	31 (12)	22 (3)	*Plantago media*	13 (5)	13 (5)
Centaurea rhenana	2 (1)	1 (0)	***Potentilla arenaria***	1 (1)	0 (0)
Centaurea scabiosa	24 (16)	9 (1)	***Potentilla heptaphylla***	4 (4)	0 (0)
Cirsium acaule	2 (2)	0 (0)	*Primula veris*	5 (4)	1 (0)
Cirsium eriophorum	9 (4)	7 (2)	*Prunella grandiflora*	3 (3)	1 (1)
Coronilla varia	42 (8)	37 (3)	***Salvia nemorosa***	4 (4)	0 (0)
Dianthus carthusianorum	1 (1)	2 (2)	*Salvia pratensis*	4 (2)	2 (0)
Eryngium campestre	9 (8)	3 (2)	*Salvia verticillata*	29 (11)	19 (1)
Euphorbia cyparissias	35 (20)	15 (0)	*Sanguisorba minor*	15 (12)	5 (2)
Euphrasia rostkoviana	4 (4)	2 (2)	*Scabiosa ochroleuca*	23 (10)	14 (1)
Festuca rupicola	37 (16)	22 (1)	*Scorzonera hispanica*	1 (0)	1 (0)
Fragaria viridis	37 (10)	29 (2)	*Stachys recta*	18 (12)	8 (2)
Galium verum	26 (9)	19 (2)	*Tanacetum corymbosum*	4 (3)	1 (0)
Gentiana cruciata	7 (2)	6 (1)	***Thymus praecox***	2 (2)	0 (0)
Genista tinctoria	4 (2)	2 (0)	*Tragopogon pratensis*	4 (1)	3 (0)
Hieracium pilosella	9 (6)	4 (1)	*Trifolium medium*	23 (13)	11 (1)
Inula hirta	3 (3)	0 (0)	***Trifolium montanum***	1 (1)	0 (0)
Inula salicina	37 (8)	29 (0)	*Veronica teucrium*	3 (2)	1 (0)

Table 2. List of traits involved in the study sorted in three groups according to what type of limitation they represent. EIV = Ellenberg indicator value.

Trait group	Variable	Units	Minimum	Mean	Maximum	SD
Seed dispersal traits	Seed weight	log(mg)	-3.31	-0.04	1.43	0.84
	Dispersal distance	log(m)	-0.56	0.29	1.18	0.32
	Exozoochory	%	0.0	18.7	80.0	22.8
	Endozoochory	%	22.2	70.8	100.0	19.9
Species habitat requirements	EIV Light	ordinal classes	6	7.3	9	0.7
	EIV Temperature	ordinal classes	5	6.0	7	0.6
	EIV Continentality	ordinal classes	2	4.4	7	1.2
	EIV Moisture	ordinal classes	2	3.7	7	0.9
	EIV Soil reaction	ordinal classes	5	7.8	9	0.9
	EIV Nutrients	ordinal classes	1	2.9	6	0.9
Availability of seeds	Frequency in grasslands	%	2.0	41.3	92.0	26.8

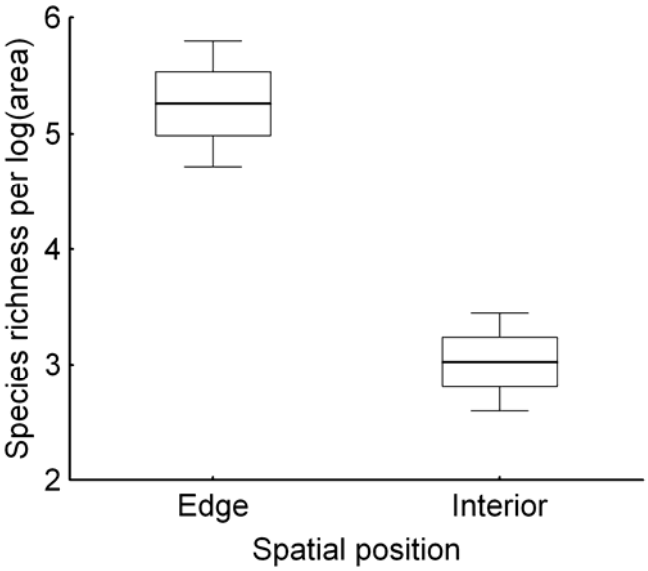


Figure 1. Differences in species richness of interiors and edges of abandoned fields. Graph shows means (bold lines) ± SE (boxes) ±1.96 SE (whiskers).

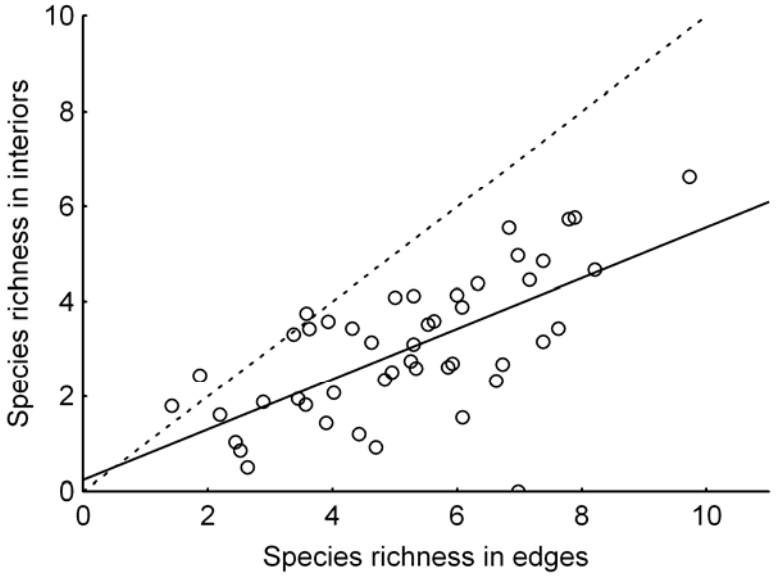


Figure 2. Species richness in edges and interiors of abandoned fields. Solid line denotes real regression line; dashed line denotes 1:1 relationship. Species richness is expressed as number of target species per log(area).

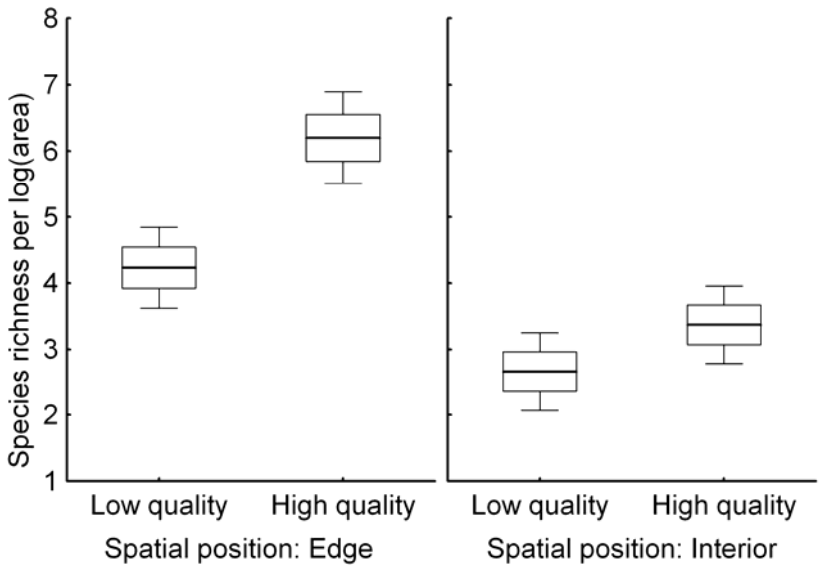


Figure 3. The effect of source quality of neighbouring habitat on species richness of whole edge segments and interiors. Graph shows means (bold lines) ± SE (boxes) ±1.96 SE (whiskers). The relationship was significant in edges ($F_{1,45} = 14.936$, $P < 0.001$) and insignificant in interiors ($F_{1,45} = 2.759$, $P = 0.104$). Source quality was assessed as follows: Dry grasslands, extensively managed gardens or orchards represented habitats of high quality and abandoned fields, intensively managed gardens or meadows, fields, forests, shrubs and built-up areas represented habitats of low quality.

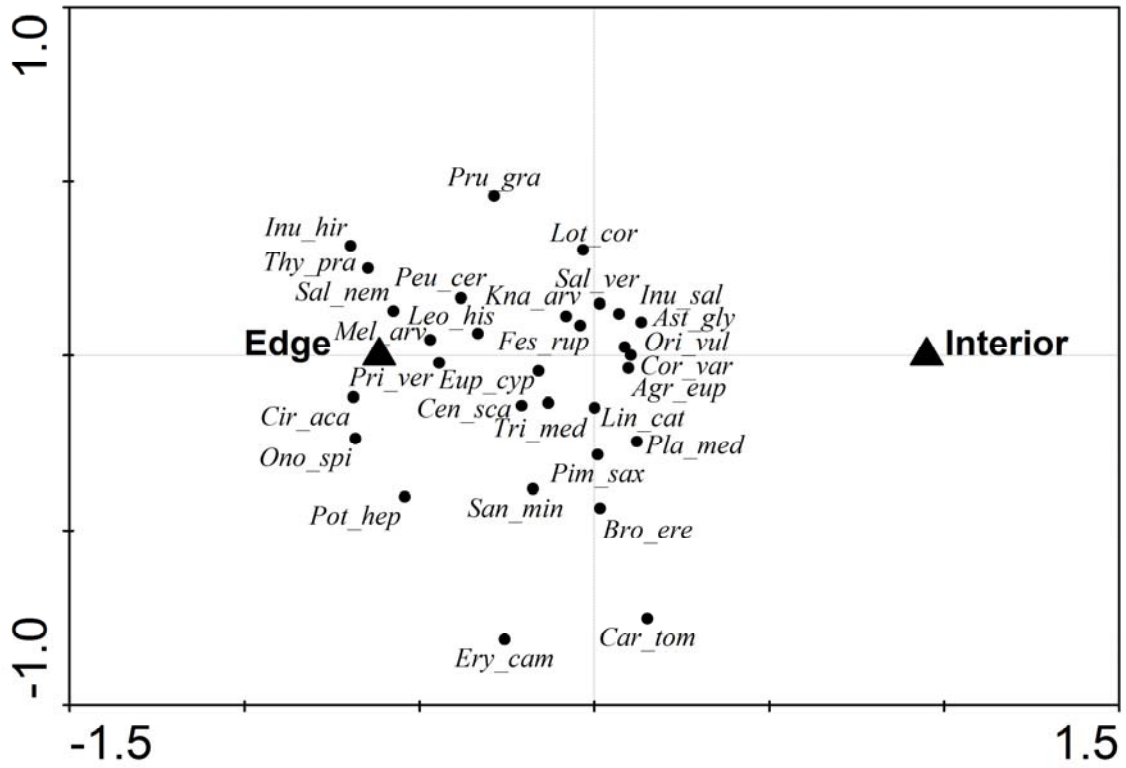


Figure 4. Species composition of edges and interiors. First (canonical) axis explained 4.2% of variation in species data, second (unconstrained) axis explained 8%. Species abbreviations consisted of first three letters of genus and species names (see Table 1). Species most on the right were found predominantly in edges whereas species in the centre were found approximately equally often in edges and interiors. None of the species occurred preferably in interiors. Only 30 species contributing most to overall variability are shown.

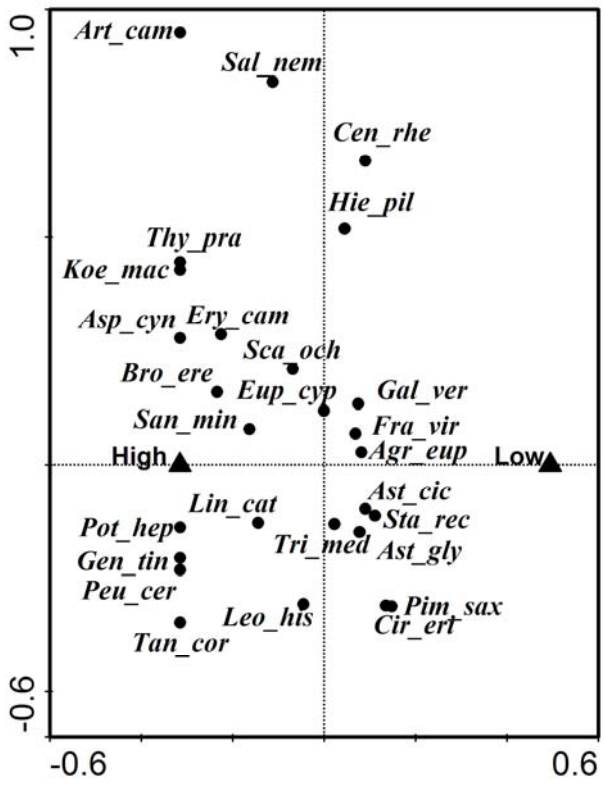


Figure 5. Species composition according to increasing source quality. First (canonical) axis explained 4.3% and second axis 8.5% of variation in species data. Source quality was assessed as follows: Dry grasslands, extensively managed gardens or orchards represented habitats of high quality and abandoned fields, intensively managed gardens or meadows, fields, forests, shrubs and built-up areas represented habitats of low quality. Species abbreviations consisted of first three letters of genus and species names (see Table 1). Analysis was performed using whole edges without further division. Only 27 species contributing most to overall variability are shown.

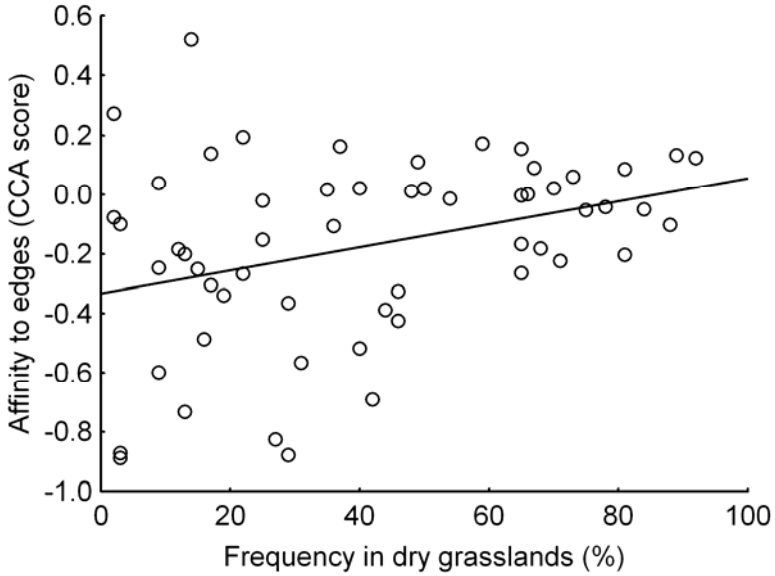
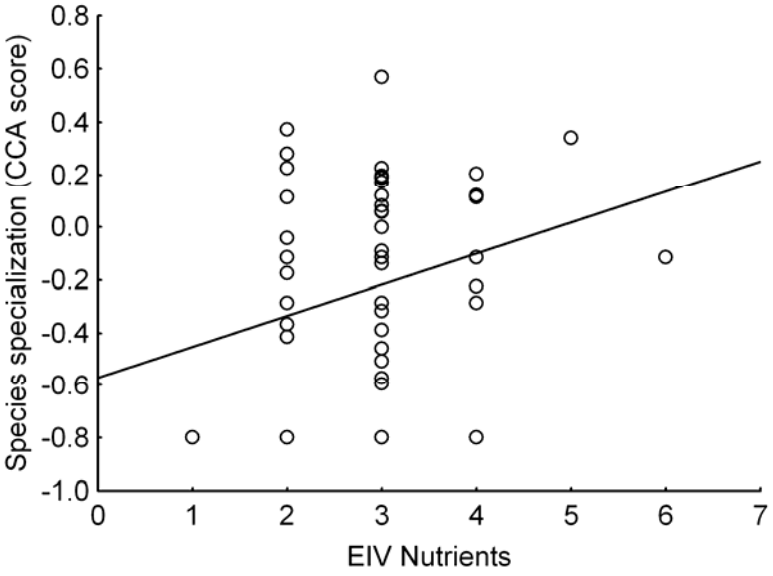
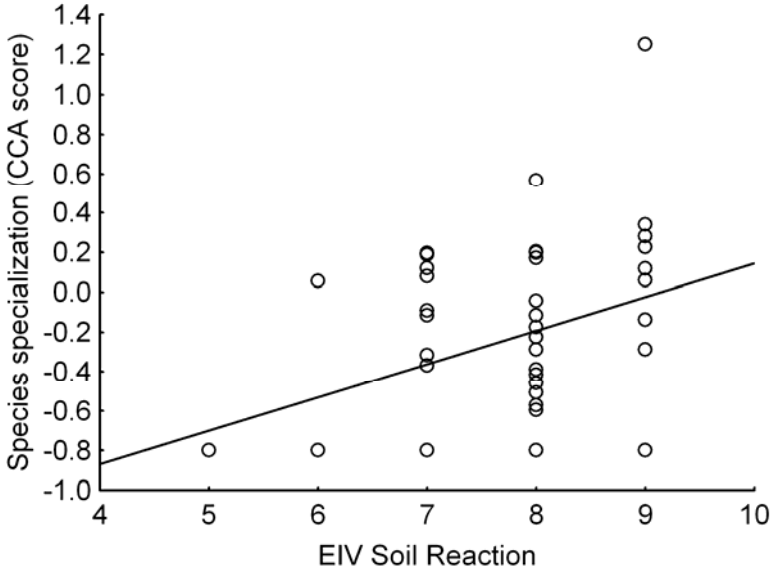


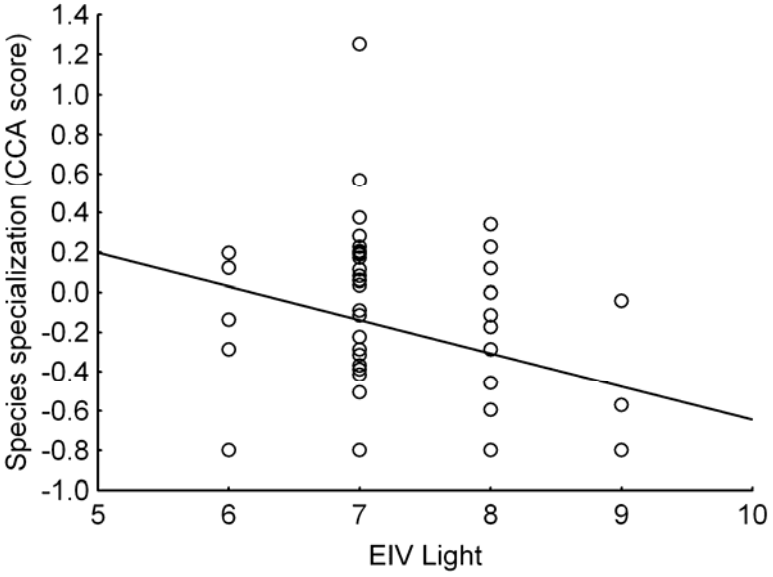
Figure 6. Relationship between species frequency in dry grasslands and affinity to field edges. As a measure of species affinity to field edges we used scores at first (canonical) axis from CCA with spatial position (edge/interior) used as explanatory variable. Negative scores indicate species were found predominantly in edges whereas species with scores around and slightly above zero were found approximately equally often in edges and interiors (see also Figure 4).


Species specialization (CCA score)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
0
1
2
3
4
5
6
7
EIV Nutrients

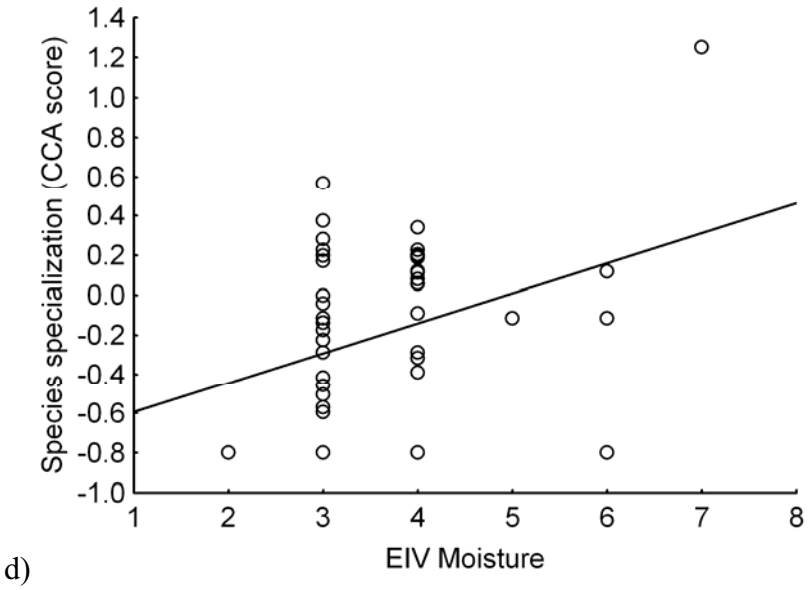
a)


Species specialization (CCA score)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
4
5
6
7
8
9
10
EIV Soil Reaction

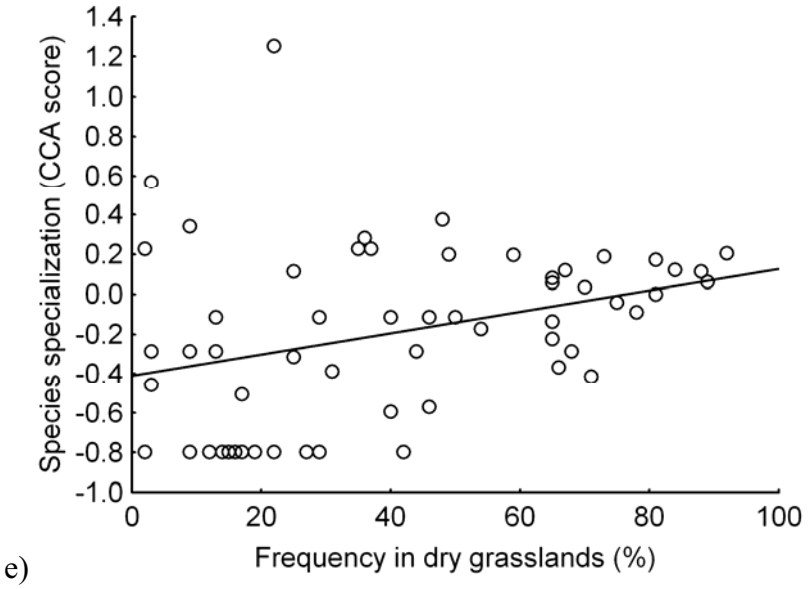
b)


Species specialization (CCA score)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
5
6
7
8
9
10
EIV Light

c)



d)



e)

Figure 7. Traits significantly related to differences in species composition among edges neighbouring with either high or low quality habitat. As a measure of species specialization we used scores at first (canonical) axis from CCA with habitat quality used as explanatory variable. Negative scores indicate species were found predominantly in edges neighbouring with high quality habitats whereas species with scores around and slightly above zero were found approximately equally often in edges neighbouring with high or low quality habitat (see also Figure 5).

References

Bartha, S., Meiners, S.J., Pickett, S.T.A., & Cadenasso, M.L. (2003) Plant colonization windows in a mesic old field succession. *Applied Vegetation Science*, **6**, 205-212.

Bates, D., Maechler, M., & Bolker, B. (2011) lme4: Linear mixed-effects models using S4 classes.

Buisson, E., Dutoit, T., Torre, F., Romermann, C., & Poschlod, P. (2006) The implications of seed rain and seed bank patterns for plant succession at the edges of abandoned fields in Mediterranean landscapes. *Agriculture Ecosystems & Environment*, **115**, 6-14.

Cousins, S.A.O. (2009) Landscape history and soil properties affect grassland decline and plant species richness in rural landscapes. *Biological Conservation*, **142**, 2752-2758.

Cramer, V.A., Hobbs, R.J., & Standish, R.J. (2008) What's new about old fileds? Land abandonment and ecosystem assembly. *Trends in Ecology & Evolution*, **23**, 104-112.

Diekmann, M. & Dupré, C. (1997) Acidification and eutrophication of deciduous forests in northwestern Germany demonstrated by indicator species analysis. *Journal of Vegetation Science*, **8**, 855-864.

Ellenberg, H. (1988) *Vegetation ecology of Central Europe*, 4th edn. Cambridge University Press.

Ellenberg, H., Weber, H., Düll, R., Wirth, V., Werner, W., & Paulissen, D. (1992) *Zeigerwerte der Gefassplanzen Mitteleuropas*, 3nd edn. Erich Goltze KG, Gottingen.

Emanuelsson, U. (2008) Semi-natural grasslands in Europe today. *Grassland Science in Europe*, **13**, 3-8.

Eriksson, O. (1996) Regional dynamics of plants: A review of evidence for remnant, source-sink and metapopulations. *Oikos*, **77**, 248-258.

Eriksson, O., Cousins, S.A.O., & Bruun, H.H. (2002) Land-use history and fragmentation of traditionally managed grasslands in Scandinavia. *Journal of Vegetation Science*, **13**, 743-748.

Eriksson, O. & Jakobsson, A. (1998) Abundance, distribution and life histories of grassland plants: a comparative study of 81 species. *Journal of Ecology*, **86**, 922-933.

ESRI (2006) ArcGIS 9.2. Environmental Systems Research Institute. Redlands.

Foster, B.L., Murphy, C.A., Keller, K.R., Aschenbach, T.A., Questad, E.J., & Kindscher, K. (2007) Restoration of prairie community structure and ecosystem function in an abandoned hayfield: A sowing experiment. *Restoration Ecology*, **15**, 652-661.

Chýlová, T. & Münzbergová, Z. (2008) Past land use co-determines the present distribution of dry grassland plant species. *Preslia*, **80**, 183-198.

Janssens, F., Peeters, A., Tallowin, J.R.B., Bakker, J.P., Bekker, R.M., Fillat, F., & Oomes, M.J.M. (1998) Relationship between soil chemical factors and grassland diversity. *Plant and Soil*, **202**, 69-78.

Jongepierová, I., Jongepier, J.W., & Klimeš, L. (2004) Restoring grassland on arable land: an example of a fast spontaneous succession without weed-dominated stages. *Preslia*, **76**, 361-369.

Knappová, J., Hemrová, L., & Münzbergová, Z. (2012) Colonization of central European abandoned fields by dry grassland species depends on the species richness of the source habitats: a new approach for measuring habitat isolation. *Landscape Ecology*, **27**, 97-108.

Lepš, J. & Šmilauer, P. (2003) *Multivariate analysis of ecological data using CANOCO* Cambridge University Press, Cambridge.

Luoto, M., Rekolainen, S., Aakkula, J., & Pykala, J. (2003) Loss of plant species richness and habitat connectivity in grasslands associated with agricultural change in Finland. *Ambio*, **32**, 447-452.

Maurer, K., Durka, W., & Stocklin, J. (2003) Frequency of plant species in remnants of calcareous grassland and their dispersal and persistence characteristics. *Basic and Applied Ecology*, **4**, 307-316.

Münzbergová, Z. (2004) Effect of spatial scale on factors limiting species distributions in dry grassland fragments. *Journal of Ecology*, **92**, 854-867.

Münzbergová, Z. & Herben, T. (2005) Seed, dispersal, microsite, habitat and recruitment limitation: identification of terms and concepts in studies of limitations. *Oecologia*, **145**, 1-8.

Murray, B.R., Thrall, P.H., Gill, A.M., & Nicotra, A.B. (2002) How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecology*, **27**, 291-310.

Öster, M., Ask, K., Cousins, S.A.O., & Eriksson, O. (2009a) Dispersal and establishment limitation reduces the potential for successful restoration of semi-natural grassland communities on former arable fields. *Journal of Applied Ecology*, **46**, 1266-1274.

Öster, M., Ask, K., Römermann, C., Tackenberg, O., & Eriksson, O. (2009b) Plant colonization of ex-arable fields from adjacent species-rich grasslands: The importance of dispersal vs. recruitment ability. *Agriculture Ecosystems & Environment*, **130**, 93-99.

Ozinga, W.A., Schaminee, J.H.J., Bekker, R.M., Bonn, S., Poschlod, P., Tackenberg, O., Bakker, J., & van Groenendael, J.M. (2005) Predictability of plant species composition from environmental conditions is constrained by dispersal limitation. *Oikos*, **108**, 555-561.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ruprecht, E. (2006) Successfully recovered grassland: A promising example from Romanian old-fields. *Restoration Ecology*, **14**, 473-480.

Ter Braak, C.J.F. & Šmilauer, P. (2002) CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca, NY, USA.

Tremlová, K. & Münzbergová, Z. (2007) Importance of species traits for species distribution in fragmented landscapes. *Ecology*, **88**, 965-977.

Turnbull, L.A., Coomes, D., Hector, A., & Rees, M. (2004) Seed mass and the competition/colonization trade-off: competitive interactions and spatial patterns in a guild of annual plants. *Journal of Ecology*, **92**, 97-109.

Tutin T.G., Heywood V.H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M. and Webb D.A., Editors. 1964-1980. *Flora Europaea* **Vols. 1–5**, Cambridge: Cambridge University Press.

Walker, K.J., Stevens, P.A., Stevens, D.P., Mountford, J.O., Manchester, S.J., & Pywell, R.F. (2004) The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation*, **119**, 1-18.

SUPPORTING INFORMATION

Appendix S1 *Description of plant traits used in the study..*

Table S1 *Results of models comparing mean Ellenberg indicator values (EIVs) between edges and interiors.*

Table S2 *Correlations among species traits, species affinity to field edges and species specialization to high quality habitat.*

Appendix S1. Description of plant traits used in the study

As a proxy of species dispersal abilities, information on seed weight, dispersal distance, attachment ability and rate of endozoochory was assembled for each species (Tremlová & Münzbergová, 2007, D. Průchová, unpublished data). Seed weight was estimated by weighing five groups of 10 seeds from three source populations (150 seeds in total). Seed weight was log transformed prior analyses. Dispersal distance *(D)* was calculated using the simple formula (Soons & Heil, 2002) $D = w*h / t$, where w is wind speed ($m.s^{-1}$), h is the release height (m) and t is terminal velocity ($m.s^{-1}$) of the species. We used the maximum daily mean wind speed over June and July, as detected by the Czech Hydrometeorological Institute at the nearby meteorological station in Doksany from 2005 to 2010 (9.6 m.s-1) as the wind speed w. Release height h (Kleyer *et al.*, 2008) was measured for ten randomly chosen individuals within three populations (30 individuals in total). Terminal velocity t was measured using dropping method (Jongejans & Schippers, 1999). Ten seeds from three populations (30 seeds in total) were released from 2 m height; each seed was released three times. Attachment ability, used as an estimate of the ability to disperse via exozoochory, was assessed by gently placing a piece of sheep fur over a tray containing 100 seeds, removing it, shaking ten times and counting the number of attached seeds (Münzbergová, 2004). This procedure was repeated with four sets of seeds for each species (400 seeds in total) and mean value was then used for each species. Rate of endozoochory express the mean percentage seeds having survived simulated digestion in comparison to the control. The simulation of ingestion and digestion includes a mechanical treatment representing chewing and a chemical treatment standing for seed digestion in the abomasum (Kleyer *et al.*, 2008). It was measured for each species using five sets of 150 seeds originating from three populations (2250 seeds in total).

As an indirect evidence for species habitat requirements, we used data on Ellenberg indicator values (Ellenberg *et al.*, 1992) for light, temperature, continentality, moisture, soil reaction and nutrients. The values are simple ordinal classes indicating optima of realized ecological niche along a gradient. The indicator values were developed mainly on the basis of field experience and reflect the ecological behaviour of species (Diekmann & Dupré, 1997).

To express availability of seeds in the landscape, we used species frequency in grasslands. It was assessed as proportion of all source grasslands (from total 339) where the species was present (Knappová *et al.*, 2012).

References

Diekmann, M. & Dupré, C. (1997) Acidification and eutrophication of deciduous forests in northwestern Germany demonstrated by indicator species analysis. *Journal of Vegetation Science*, **8**, 855-864.

Ellenberg, H., Weber, H., Düll, R., Wirth, V., Werner, W., & Paulissen, D. (1992) *Zeigerwerte der Gefassplanzen Mitteleuropas*, 3nd edn. Erich Goltze KG, Gottingen.

Jongejans, E. & Schippers, P. (1999) Modeling seed dispersal by wind in herbaceous species. *Oikos*, **87**, 362-372.

Kleyer, M., Bekker, R.M., Knevel, I.C., Bakker, J.P., Thompson, K., Sonnenschein, M., Poschlod, P., van Groenendael, J.M., Klimeš, L., Klimešová, J., Klotz, S., Rusch, G.M., Hermy, M., Adriaens, D., Boedeltje, G., Bossuyt, B., Dannemann, A., Endels, P., Goetzenberger, L., Hodgson, J.G., Jackel, A.K., Kuehn, I., Kunzmann, D., Ozinga, W.A., Roemermann, C., Stadler, M., Schlegelmilch, J., Steendam, H.J., Tackenberg, O., Wilmann, B.,

Cornelissen, J.H.C., Eriksson, O., Garnier, E., & Peco, B. (2008) The LEDA Traitbase: a database of life-history traits of the Northwest European flora. *Journal of Ecology*, **96**, 1266-1274.

Knappová, J., Hemrová, L., & Münzbergová, Z. (2012) Colonization of central European abandoned fields by dry grassland species depends on the species richness of the source habitats: a new approach for measuring habitat isolation. *Landscape Ecology*, **27**, 97-108.

Münzbergová, Z. (2004) Effect of spatial scale on factors limiting species distributions in dry grassland fragments. *Journal of Ecology*, **92**, 854-867.

Soons, M.B. & Heil, G.W. (2002) Reduced colonization capacity in fragmented populations of wind-dispersed grassland forbs. *Journal of Ecology*, **90**, 1033-1043.

Tremlová, K. & Münzbergová, Z. (2007) Importance of species traits for species distribution in fragmented landscapes. *Ecology*, **88**, 965-977.

Table S1. Results of models comparing mean Ellenberg indicator values (EIVs) between edges and interiors. Mixed effect models were fitted with spatial position (edge/interior) as fixed factor and field code as random term. Direction denotes whether higher values were achieved in edges or interiors.

	$F_{1,45}$	*P*	Direction
EIV Light	3.77	0.058	I < E
EIV Temperature	**4.71**	**0.035**	**I < E**
EIV Continentality	0.16	0.692	I > E
EIV Moisture	**7.31**	**0.010**	**I > E**
EIV Nutrients	0.98	0.327	I > E
EIV Soil Reaction	0.18	0.672	I > E

Table S2. Correlations among species traits, species affinity to field edges and species specialization to high quality habitat. Significant values ($P < 0.05$) are in bold. As a measure of species affinity to field edges we used scores at first (canonical) axis from CCA with spatial position (edge/interior) used as explanatory variable. Negative scores indicate species were found predominantly in edges whereas species with scores around and slightly above zero were found approximately equally often in edges and interiors. As a measure of species specialization we used scores at first (canonical) axis from CCA with habitat quality used as explanatory variable. Negative scores indicate species were found predominantly in edges neighbouring with high quality habitats whereas species with scores around and slightly above zero were found approximately equally often in edges neighbouring with high or low quality habitat. EIV = Ellenberg indicator value.

	Frequency in dry grasslands (%)	EIV Light	EIV Temperature	EIV Continentality	EIV Moisture	EIV Soil Reaction	EIV Nutrients
Affinity to edges (CCA score)	**0.337**	-0.090	-0.234	0.003	0.062	-0.128	0.013
Species specialization (CCA score)	**0.335**	**-0.288**	0.036	0.104	**0.318**	**0.340**	**0.277**
Frequency in dry grasslands (%)	1.000	-0.127	-0.151	0.006	0.018	0.041	0.146
EIV Light	-0.127	1.000	0.106	0.125	-0.179	-0.052	-0.204
EIV Temperature	-0.151	0.106	1.000	**0.496**	0.045	0.090	0.108
EIV Continentality	0.006	0.125	**0.496**	1.000	-0.217	0.206	-0.197
EIV Moisture	0.018	-0.179	0.045	-0.217	1.000	0.032	0.255
EIV Soil Reaction	0.041	-0.052	0.090	0.206	0.032	1.000	0.111
EIV Nutrients	0.146	-0.204	0.108	-0.197	0.255	0.111	1.000